DINABANDHU ANDREWS COLLEGE
NAAC Accredited
(Government Sponsored)
Estd. 1956

Ref. No. ..... Date .....

NAME OF THE COLLEGE/ INSTITUTION- Achrya

Prafulla Chandra College

DATE OF MOU- 14/04/2021

PURPOSE OF MOU- Both the Parties are pleased to enter upon an agreement to establish ties of academic
cooperation in order to contribute to the achievement of their overall goals as institutions
through following but not limited to:

1. Joint teaching and research projects.
2. Visit, training and exchange of faculty, staff and students
3. Joint educational/vocational courses.
4. Special short term academic programme an workshops.
5. Co-hosting and participation in conferences, seminars.

ACTIVITIES- Faculty Exchange Programme & research

collabortion

Baishnabghata, P.O. Garia, Dist. 24 Parganas (S), Kolkata - 700 084
Ph. : (033) 2430 4377, e-mail : dacprincipal@gmail.com. website : www.dacollege.org

ACTIVITY DETAILS UNDER MoU

1. Name of the Collaborating Colleges: Dinabandhu Andrews College and Acharya Prafulla Chandra College
2. Date of execution of MoU: 14.04.2021

Session	Nature of activity	Venue	Topic	Number of Students/ Teachers participated
24.03.2022	Faculty Exchange Programme	Dept. of Botany, Acharya Prafulla Chandra College	BOTGDSE02T Unit 7 :The art of scientific writing and its presentation	**Teacher:** Dr. Joy Sarkar Associate Professor Dinabandhu Andrews College Students (A.P.C. College) : 14
07.04.2022	Faculty Exchange Programme	Dept. of Botany, Acharya Prafulla Chandra College	BOTACOR13T Unit 4 :Carbon oxidation	**Teacher:** Dr. Joy Sarkar Associate Professor Dinabandhu Andrews College Students (A.P.C. College) : 16
06.04.2022	Faculty Exchange Programme	Dept. of Physics, Dinabandhu AndrewsCollege	Method of Images	**Teacher:** Dr.Ananda Sarkar Associate Professor A.P.C. COLLEGE Students (Dinabandhu Andrews . College) : 15
20.04.2022	Faculty Exchange Programme	Dept. of Physics, Dinabandhu AndrewsCollege	Method of Images	**Teacher:** Dr. Ananda Sarkar Associate Professor A.P.C. COLLEGE Students (Dinabandhu Andrews . College) : 12
02.08.2021	Publication or Research Collaboration	Department of Botany, Dinabandhu Andrews College & Acharya Prafulla Chandra College	A comprehensivereview of variouscategories of facemasks resistant to Covid-19 DOI: https://doi.org/10.1016/j.cegh.2021.100835	**Teacher** Dr. Joy Sarkar Associate Professor Dinabandhu Andrews College & Pallab Chakraborty (Student A.P.C. College)

Principal
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Session	Nature of activity	Venue	Topic	Number of Students/ Teachers participated
19.03.2022	Publication or Research Collaboration	Department of Botany, Dinabandhu Andrews College & Acharya Prafulla Chandra College	Mucormycosis: A new threat to Coronavirus disease 2019 with special emphasis onIndia DOI: https://doi.org/10.1016/j.cegh.2022.101013	**Teacher** Dr. Joy Sarkar Associate Professor Dinabandhu Andrews College & Pallab Chakraborty (Student A.P.C. College)
03.02.2022	Publication or Research Collaboration	Department of Botany, Dinabandhu Andrews College & Acharya Prafulla Chandra College	Clinical aspects and presumed etiology of multisystem inflammatory syndrome in children (MIS-C): A review DOI: https://doi.org/10.1016/j.cegh.2022.100966	**Teacher** Dr. Joy Sarkar Associate Professor Dinabandhu Andrews College & Pallab Chakraborty (Student A.P.C. College)
30.04.2021	Publication or Research Collaboration	Department of Botany, Dinabandhu Andrews College & Acharya Prafulla Chandra College	Comparative review on left- handed Z-DNADOI: 10.52586/4922	**Teacher** Dr. Joy Sarkar Associate Professor Dinabandhu Andrews College & Pallab Chakraborty (Student A.P.C. College)
27.01.2022	Publication or Research Collaboration	Department of Botany, Dinabandhu Andrews College & Acharya Prafulla Chandra College	Green synthesis of copper/copper oxide nanoparticlesand their applications: a review DOI: https://doi.org/10.1080/17518253.2022.2025916	**Teacher** Dr. Joy Sarkar Associate Professor Dinabandhu Andrews College & Pallab Chakraborty (Student A.P.C. College)

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Date .....

Date:

TO WHOM IT MAY CONCERN

This is to certify that the following activities have been conducted under **Teachers' Exchange Programme** as per the **MoU** signed between Acharya Prafulla Chandra College and Dinabandhu Andrews College on 14.04.2021.

Teacher Exchange Programme for the session 2022-23

Date	Name of Teacher	Department	Topic taught	Course/ Year/ Semester	Duration
06.04.2022	Dr. Ananda Sarkar Associate Professor	Dept. of Physics, Acharya Prafulla Chandra College	Method of Images	B. Sc. Semester-II	2 hrs
20.04.2022	Dr. Ananda Sarkar Associate Professor	Dept. of Physics, Acharya Prafulla Chandra College	Method of Images	B. Sc. Semester-II	2 hrs

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Ref. No. ..... *Date* .....

List of Students who attended class on (06.04.2022, 20.04.2022)

Sl. No.	Department	Name
1	Physics	Reshmi Chakraborty
2	Physics	Swastika Halder
3	Physics	Swastika Giri
4	Physics	Swagata Surya Mahapatra
5	Physics	Ankur Das
6	Physics	Sahin Alam Sk.
7	Physics	Adreesh Bairagi
8	Physics	Rajarshri Sen
9	Physics	Mrinmoy Mondal
10	Physics	Satyadeep Mondal

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Date : 11/05/2023

TO WHOM IT MAY CONCERN

This is to certify that the following activities have been conducted under **Teachers Exchange Programme** as per the **MoU** signed between Acharya Prafulla Chandra College and Dinabandhu Andrews College on 14.04.2021.

Teacher Exchange Programme for the session 2022-23

Date	Name of Teacher	Department	Topic taught	Course/ Year/ Semester	Duration
24.03.2022	Dr. Joy Sarkar Associate Professor	Dept. of Botany, Dinabandhu Andrews College	BOTGDSE02T Unit 7 :The art of scientific writing and its presentation	B.Sc Semester-IV	2 hrs
7.04.2022	Dr. Joy Sarkar Associate Prtofessor	Dept. of Botany, Dinabandhu Andrews College	BOTACOR13T Unit 4 :Carbon oxidation	B.Sc. Semester-VI	2 hrs

11/5/23

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Registration Number	Name
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1012011100272	MAINAK DAS
1012011100279	SUBHADIP CHANDRA MAHAJAN
1012011400265	SUBHAM CHATTERJEE
1012011400268	SWAGATA HALDAR
1012011400275	SNEHASHIS BHATTACHARYYA
1012011400276	SOURAV KANGSA BANIK
1012015400266	SAMBIT CHOWDHURY
1012021100269	MUNMUN SARKAR
1012021100274	SUDESHNA MONDAL
1012021400270	TIYAS CHAKRABORTY
1012021400273	SUSMITA DUTTA
1012021400277	ARITRA KARMAKAR
1012021400278	ARPITA SAHA

List of Students who attended class on (7.04.2022)
Dept. of Botany SEM VI

Registration Number	Name
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1011911101497	SHUBHADEEP BAIN
1011911101501	NIRUPOM ROY
1011911401491	SRIJAN DHAR
1011911601500	ANIKET BISWAS
1011921101488	SHARNALI SARKAR
1011921401484	SRIJITA SARKAR
1011921401487	SNEHA SOM CHOWDHURY
1011921401489	SHRABANTI KUNDU
1011921401494	MANJEEMA ROY
1011921401495	PREITY MONDAL
1011921401498	ANKITA SARKAR
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Review article

A comprehensive review of various categories of face masks resistant to Covid-19

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ABSTRACT

The science about the usage of face masks by the common public to avert COVID-19 transmission is proceeding swiftly. A primary route of transmission of COVID-19 is probably through small respiratory droplets, and it is transmissible from asymptomatic and pre-symptomatic individuals. According to the World Health Organization, wearing a mask in public can help reduce the transmission of the COVID-19 virus. Different categories and types of masks and their usage are reviewed in this work. In a nutshell, this review work elucidates the aspects of utilizing the various face masks along with all possibilities to fight against the ongoing pandemic of COVID-19.

1. Introduction

World Health Organization (WHO), announced on January 30, 2020, a Public Health Emergency of International Concern (PHEIC) in response to the emersion of a novel coronavirus in Wuhan, China. Later, on March 11, 2020, WHO announced COVID-19 to be a pandemic, it is the 2nd pandemic of the 21st century after the pandemic of 2009 caused by influenza A H1N1.[1,2]

In the declaration by WHO on February 11, 2020, the Coronavirus disease (COVID-19) is caused by the virus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is genetically related to the coronavirus responsible for the SARS outbreak of 2003.[3]

As of August 29, 2020, more than 24.7 million cases have been reported across 188 countries and territories, causing more than 837,000 deaths with a recovering of 16.2 million people. In this case, proactive infection monitoring criteria have been executed in hospital set-ups.[4,5]

To control the further outbreak of pandemic disease COVID-19 among people, WHO provided some guidelines and instructions to the general public to follow such as the continuous practice of hand hygiene, maintaining social distancing, wearing a mask in public, avoiding social gatherings and practising self-isolation and home quarantine. In addition, with the guidelines of WHO, the authorities also implemented some rules such as quarantine and testing of all travellers, closing and regulating the city and country borders, along with massive testing for case detection by RT-PCR (reverse-transcription polymerase chain reaction) technique. Later, stay at home order, lockdowns, home isolation, cancellation of mass gatherings and prohibiting traveling were acquired to several degrees and at various time points in several countries to alleviate the threat of community transmission. It is unclear when the outbreak will end, and there are no known vaccines or antiviral therapies that are 100% effective against the coronavirus.[3,5] Though there has been working on immunization and numerous vaccines have been created so far, none of them can guarantee 100 % efficacy against SARS-CoV-2.[6–8]

To handle the ongoing COVID -19 pandemic situation, the US Centers for Disease Control and Prevention (CDC) had recommended the public to put on face masks. Many Asian countries, which have had greater experience with new coronavirus infections, use public masks significantly more frequently. Face Masks have been recommended as a primary potent tool to control the COVID-19 outbreak in China.[9] However, the World Health Organization (WHO) advises that face masks should only be worn by individuals caring for patients with suspected COVID-19 or those who are actively sneezing or coughing.[10]

Many governments around the world have introduced policies that recommend the wearing of masks to slow down the expanse of COVID-19. Mandatory use and enforcement vary globally. While several

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countries have passed legislation requiring the use of masks, others, like China, India, Japan, South Korea, and Taiwan, have issued more precise guidelines.[11]

With the exponential spread and emergence of COVID 19, the usage and utility of masks and respirators for the common people have been advised by the government.[10] The US CDC has recommended that the usage of Medical masks, surgical masks, cloth masks, fabric masks, and extended use of respirators can be deliberated with proper caution. Medical masks i.e., N95 respirators and surgical masks are prioritized for healthcare professionals which appears to protect against the virus.[12] Countries, like Italy and Mexico, provided single-use masks for the common public upon mandating their use.[11] If medical masks, surgical masks or respirators are not available then the general public can use homemade cloth masks that provide lesser protection but still afford the efficacy.[13] Japan offered cotton masks without imposing mandatory use, whereas the Czech Republic and the United Kingdom urged citizens to use reusable masks.[11] There is not yet enough data to determine which combination of mask policies are most effective in slowing the expanse of infection. However, it is advised to wear reusable face coverings or masks for the general public as an alternative to the single use face mask or surgical face masks used in healthcare sector. The usage of reusable cloth mask are effective in combating virus transmission to a certain extent when combined with non-clinical interventions such as maintaining social distance and hand hygiene.[11] In one of the surveys, it is reported that 28,000 people aged between 16 and 74 years in 15 countries wore face masks because of the coronavirus outbreak. About more than 70 % of people in India, China, Italy, Japan and Vietnam wore reusable face masks to protect themselves from coronavirus outbreaks. In the USA and other countries, the percentage is 50 % and above .[9,11] The sensations during the previous five months show that mask usage was usually high (>75 %) in certain locations, such as Asia and South and Central America, whereas it was always low (25 %) in Northern Europe. In some nations, an improvement over time has been observed, which might help in evaluating the surging cases of covid-19 or mask-related requirements and guidance.[11]

These and associated data can support inform public health communications campaigns and endeavors considering mask-wearing to help slow the expanse of COVID-19 and could be borrowed to help evaluate how policies associate to practice around the world.

In this paper, we seek to elucidate the different aspects of masks. Specifically, we explore the different varieties of masks, their material composition and effectiveness in protecting the wearer from an airborne virus along with the guidelines of usage of masks and caring method of masks in detail.

We tend to provide more information to the general public about the types of masks and what mask they should choose in case of no medical or surgical mask is available.

1.1. Transmission characteristics of Covid-19

Every day, new information concerning the COVID-19's transmission emerges. It is mainly a respiratory disease and the spread of infection with this virus can range from people with mild, non-respiratory symptoms to extreme acute respiratory ailment, along with organ dysfunction, sepsis and death, while some infected people have no symptoms at all. According to recent reports, the coronavirus is transmitted among people via contact routes and respiratory droplets. Transmission can also happen through fomites in the infected person's immediate environment. Therefore, transmission can occur by direct contact with an infected person, or indirectly by contact with surfaces or objects used on or by the infected person[5,14] (Fig. 1).

The threshold for the droplet size is ranging from 5 μm to 10 μm. Droplet transmission happens when a healthy individual comes in close contact within 1 m with an infected person and is exposed to infectious respiratory droplets, through coughing, sneezing or close personal contact by mouth or nose.[15]

Airborne transmission of the COVID-19 virus may be possible in particular conditions where procedures to generate aerosols are performed. The research community has been discussing, whether the coronavirus, might furthermore circulate by aerosols in the deficiency of aerosol-generating procedures (AGPs). This is a province of strong research. As, air testing in clinical atmospheres where AGPs were not conducted, found RNA virus in some observations but not in others.[16] Nevertheless, the existence of viral RNA is never similar to replication and infection competent (viable) virus that could be transmissible and able to adequate inoculum to commence invasive infection. Few

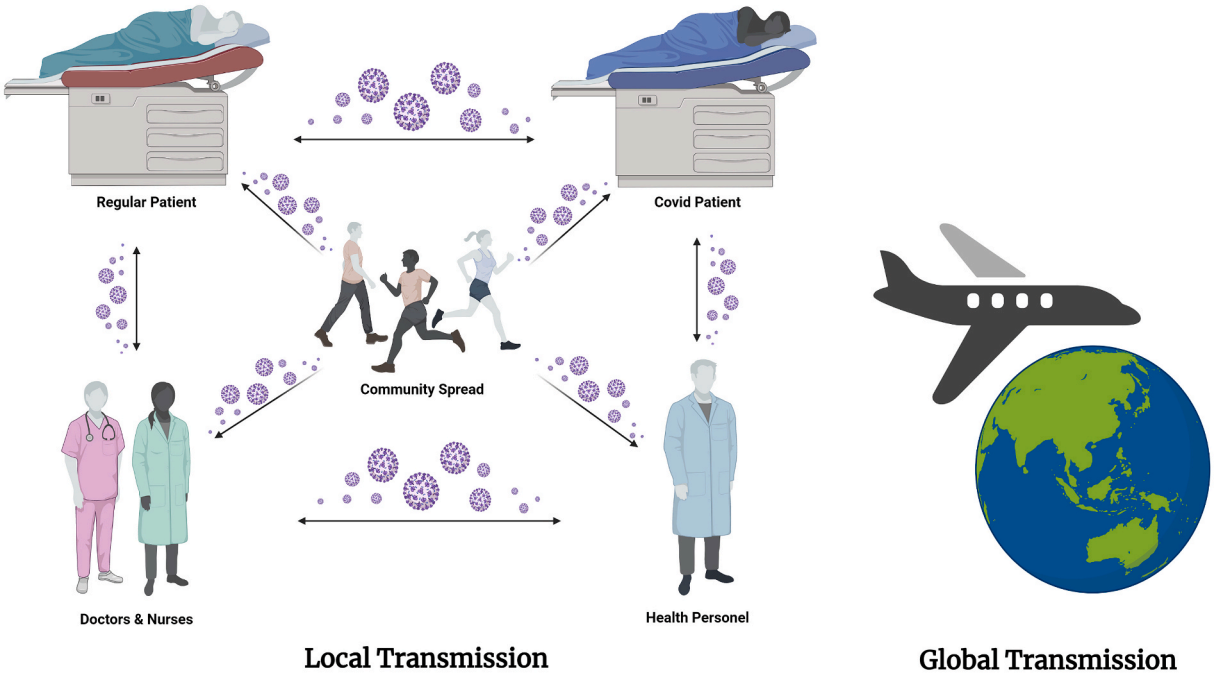

Fig. 1. Diagram shows the different modes of COVID-19 disease transmission (Created with BioRender.com).

experimental studies performed by aerobiology laboratories have found the viable virus and viral RNA, but these were experimentally induced AGP where aerosols were produced artificially using high-powered jet nebulizers and do not imitate normal human cough conditions.[17]

To address several of the acknowledged research gaps connected with AGPs and COVID-19 virus airborne propagation, high-quality research involving randomised trials in a variety of situations is required.

Recent information recommends that maximum expanse of COVID-19 is happening from symptomatic people to others in near contact, when not wearing proper PPE. Amongst the infected persons with symptoms, viral RNA can be found in their specimens', weeks afterwards the commencement of sickness. However, for moderately infected individuals, the viable virus was not discovered after 8 days after the onset of symptoms, albeit this can change for very ill patients.[18] In pre-symptomatic transmission, the infected person with no symptoms can transmit the virus to others. SARS-CoV-2 has an incubation period of 5- to 6-days, but it can last up to 14 days.[19,20] Besides that, some data indicate that many people may test positive for coronavirus, using polymerase chain reaction (PCR) testing, 1–4 days before any symptoms develop.[21]

As per reports, some individuals infected with the SARS-CoV-2 virus does not ever develop any symptoms, though they can shed the virus that may, therefore, be transmitted to other people. According to a recent systematic analysis, 6 %–42 % of infected people are asymptomatic, and most of the studies in their evaluation have limitations due to inadequate symptom reporting. Comprehensive researches on the transmission from asymptomatic people are tough to perform, but the accessible proof from contact tracing documented by the Member States implies that asymptotically infected patients are much less likely to transmit the virus than those who develop symptoms.[22]

1.2. Recommendations for wearing a face mask

There have been reports on the usage of surgical face masks on patients with pulmonary tuberculosis, which significantly reduced transmission and offer an adjunct measure for reducing TB transmission from infectious patients to healthy persons.[23,24] The surgical mask has further been revealed to disengage other human coronaviruses during coughing. In addition, a meta-analysis of randomised trials found that surgical masks and N95 respirators were equally effective in preventing influenza-like illness and laboratory-confirmed influenza among healthcare workers.[25]

The use of a mask to control a respiratory illness is a well-established method. A relevant investigation discovered that a cloth mask obstructed 96 % of viral quantity on normal when used eight inches away to wheeze from a COVID-19-infected patient.[26] It has been shown that every 10-fold increase in viral amount results in an additional 26 % increase in patient fatalities from acute infections caused by highly deadly viruses.[27]

The research focused on aerosol disclosure has found that all kinds of masks are at least somewhat beneficial at defending the wearer. Van der Sande et al. in 2008 reported that any mask can decrease aerosol exposure and are reasonably reliable over time. Researchers found that any type of mask use is likely to decrease viral exposure and infection hazard on a community grade, despite its improper fit.[28]

However, the examination of particle filtration is likely to misjudge the efficiency of masks, as the number of particles that are secreted as an aerosol is relatively small.[29] Both homemade and medical masks are considerably useful in minimizing the number of microorganisms and can reduce the spread of infection, though the surgical mask was much better and be more useful in obstructing transmission in comparison to homemade masks.[28] The scarcity of surgical masks and N95 respirators is a major issue. According to the current CDC advice, a healthy person should wear a cloth face mask in public.[30]

The importance of utilizing masks for health care persons has been detected in Chinese hospitals where, in each hospital, medical professionals wearing masks (particularly in quarantine areas) had no COVID-19 infections, instead of being around COVID-19 patients while other medical professionals had 10 or more infections in hospitals because of not wearing the mask.[31]

1.3. Categories of face masks recommended by WHO

During this COVID-19 epidemic, the usage of masks is suggested by Governments and WHO to control the further expanse of SARS-CoV-2. The usage of masks has followed various guidance from several community health organizations and governments. The WHO and other public health organizations approve that masks can inhibit the expanse of respiratory viral diseases, especially in COVID-19 case.[32]

WHO recommends various kinds of masks for use in pandemic COVID-19 (Fig. 2). These types are mentioned as below:

1.4. Categories of face masks include

1. Cloth face masks
2. Medical or surgical masks
3. Respirators:
   (i) Filtering facepiece respirator
      • NIOSH respirator filter masks
   (ii) Full-length face shield
   (iii) Self-contained breathing apparatus (SCBA)

2. Cloth face masks

A cloth face mask is inexpensive and made of everyday cotton fabric that is worn over the mouth and nose. Many health authorities are instructed to use these cloth masks for protection if medical masks are unavailable in stocks.[33]

It is solely made of several varieties of cloth material. Studies demonstrate that the efficiency of these cloth masks, when compared to the N95 mask, is less effective against the SARS-CoV-2 but they can still provide the basic protection. It does provide the user protection against the air contaminants like pollens and dust particles. Therefore, it was limited approved in case of a pandemic.

Before the coronavirus outbreak, several Asian countries, including Vietnam and China, investigated the use of cloth masks in the community and healthcare.[34,35] Cotton cloth masks were reported to be used by Health Care Workers (HCWs) in China during the outbreak of severe acute respiratory syndrome (SARS) in 2002.[36]

2.1. Types of cloth masks

Based on laboratory data and WHO's instruction, cloth masks are of three types: (a) Cloth mask 1, (b) Cloth mask 2, (c) Cloth mask 3.

Cloth mask 1 contains a latex exhalation valve, which worked better than the other two cloth masks that did not have an exhalation valve. The capability of filtration efficiencies of cloth masks 2 and 3 varied among the several PSL (polystyrene latex) sizes. Cloth masks 2 and 3 are more susceptible to penetration than cloth mask 1. Cloth mask 1, is an outstanding filtering mask with a conical or tetrahedral shape, that fits well with the general population. It also has 3 layers with a hydrophilic inner layer, filter in the middle layer and a hydrophobic outer layer whereas cloth masks 2 and 3 have simple rectangle long nooses and they do not possess three layers. Cloth mask 2 has two layers i.e. filter and hydrophobic outer layer while cloth mask 3 has only one thin layer (Fig. 3). This technique does not fit on the mannequin, allowing the leakage of a considerable percentage of components to infiltrate via the mask. When comparing filtering effectiveness and fit, cloth mask 1 outperformed the other two masks. However, the cloth mask 2 performed better when compared to cloth mask 3[37]

The most protective cloth face masks require at least three layers

Fig. 2. Diagram shows the pictorial view of different types of masks: **a.** Surgical Mask **b.** Cloth Mask **c.** Cloth Mask with Head Loop **d.** Fold Model Type K95 Mask without Respirator with Head Loop **e.** Fold Model Type N95 Mask with Respirator and Head Loop **f.** Cup Model Type N95 Mask with Respirator and Head Loop **g.** Full-Length Face Shield.

with a hydrophilic inner layer (e.g. cotton) to consume moisture from the wearer's breathing and hydrophobic outer layers (e.g. polyester).[37]

Another study demonstrated that homemade masks made of tea cloth delivered safety during short- and long-term activities.[28] Ma et al. demonstrated that while N95 respirators obstructed 99.98 % of avian influenza virus, cloth homemade masks and surgical masks were comparative 95.15 % and 97.14 %, respectively. These homemade masks were created from polyester and kitchen towels and were used in the experiment.[34,38]

3. Medical or surgical mask

During this moment of global health emergency caused by the COVID-19, WHO recommends medical or surgical masks for nurses, doctors, patients, and all hospital personnel, as well as all healthcare units, to protect themselves from COVID-19 exposure. A fluid-resistant (Type-IIR) medical face cover is utilized to keep safe against droplets. If worn by the sufferer, it will underestimate the disbandment of enormous respiratory droplets that will defend workers against both droplets and influenced by the transmission of viruses. If worn by healthcare workers it will protect against droplet communication, when within 1–2 m of the victim. Danger removal by at least 80 % is reckoned.[39] Surgical masks contain three layers: an internal tender absorbent sheet, a central polypropylene obstacle, and an exterior hydrophobic surface. This face mask delivers protection from droplets in a clinical setting. The design of the surgical masks relies on the mode; usually, the masks are three-ply (three layers) and 4 ply (four layers). This three-ply fabric is composed of a meltblown polymer, most generally polypropylene, placed between the non-woven fabric. It has 3 layers, the external layer repels water droplets, the middle layer serves as a filter and the interior layer absorbs moisture (Fig. 4a & 4b). Numerous analyses are carried out to disclose the removal of viral detection, which in turn was found to be 25-fold for coarse aerosols, 2.5 fold for neat particles. Surgical face masks deliver the fundamental option in a pandemic. It had the

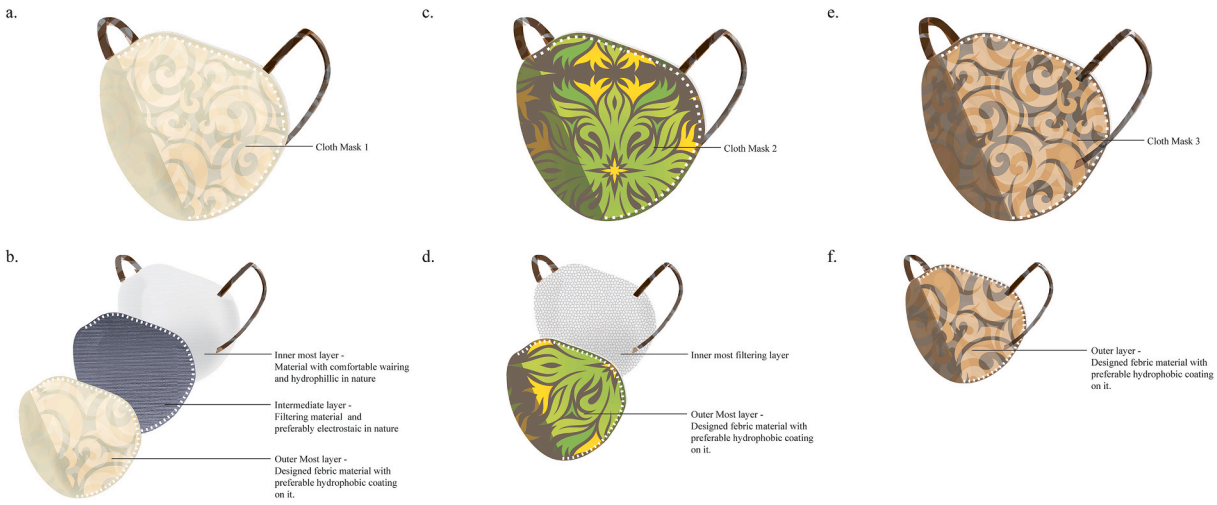

Fig. 3. Diagram shows the different types of cloth masks and their layering pattern: **a.** Cloth Mask 1 **b.** Different layering pattern of Cloth Mask 1 **c.** Cloth Mask 2 **d.** Different layering pattern of Cloth Mask 2 **e.** Cloth Mask 3 **f.** Different layering pattern of Cloth Mask 3.

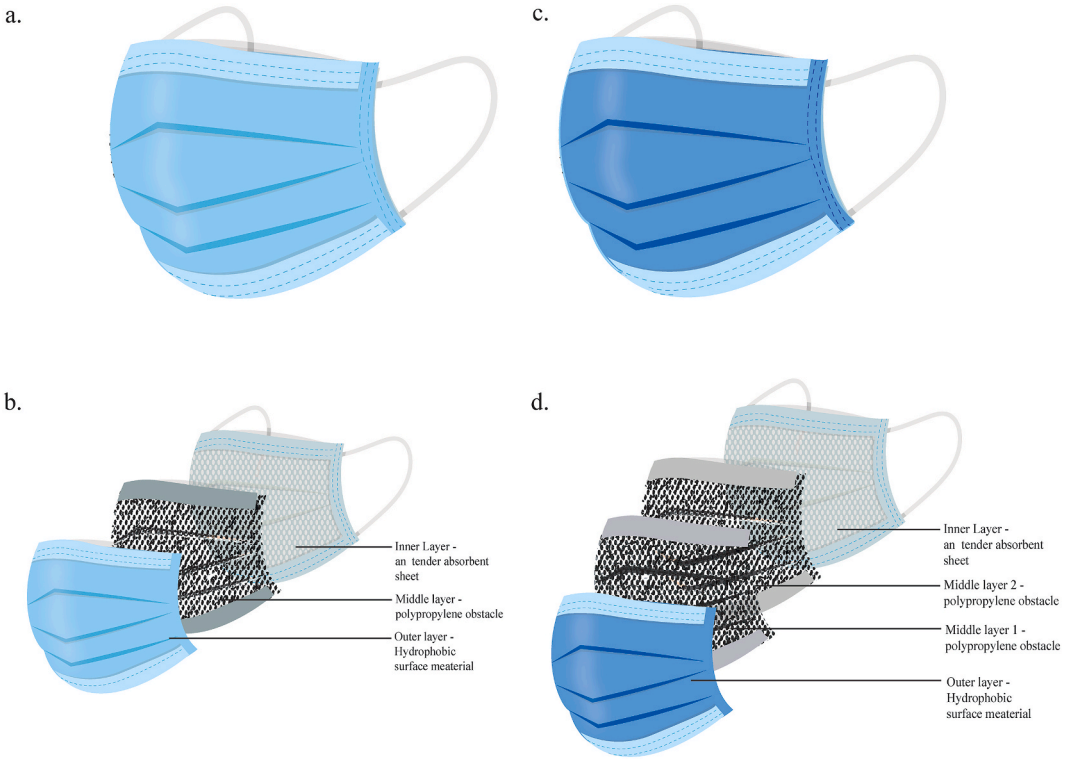

Fig. 4. Diagram shows the different types of surgical masks and their layering pattern: **a.** 3-ply surgical mask **b.** Different layering pattern of 3-ply surgical mask **c.** 4-ply surgical mask **d.** Different layering pattern of 4-ply surgical mask.

drawback of not constraining the release of minor droplets.[40]

The 4 ply surgical mask is like a 3 ply face mask along with an added additional layer with an activated carbon filter or one more filtering layer (Fig. 4c & 4d). The first layer is composed of polypropylene spun bond non-woven, second is with an active carbon filter fiber or another filtering layer. The 3rd layer is with melt-blown nonwoven fabric and the final layer is with polypropylene spun bond non-woven. They also have flexible nose strips to give extreme protection and satisfaction to the user. It also protects against odors as well as organic vapors.[40]

4. Respirators

FFP 1/2/3 or NIOSH respirators and other respirators are seal-tested respirators and they are capable to protect health care workers especially those who directly come in contact with the patients. This apparatus causes a blockage around the nose and mouth and has twisted fibres with filters. WHO instructed about respirators, described below:

(i) Filtering Face-piece Respirators:

The term filtering facepiece (FFP) is utilized in source to high-performance screening masks. Filtration is executed by a variety of

complex polypropylene microfibers and electrostatic rates. It is borrowed to filter out vapors, dust particles and infectious agents. It is primarily borrowed in the workplace having more pollutants. It has the benefit of cleansing air and cutting down the hazard of contamination of the wearer. Such mechanical filter respirators protect against the inhalation of particulates such as dust particles, droplets, and aerosols.[40]

Types of FFP Mask: There are three categories of protection. For FFP1, FFP2 and FFP3 these are 4-, 10- and 20-folds, respectively. FFP1 Filters at least 80 % of airborne particles and FFP2 Filters at least 94 % of airborne particles.[40]

The third category of FFP3 provides the longest aspect of precaution and is the only one recommended for UK healthcare locations, particularly in AGPs, such as intubation and non-invasive ventilation. They must comply with industry standards, including stringent industry tests with biological aerosols and a maximum leakage of 2 %. FFP3 respirators provide 99% protection against COVID-19 in screening elements ranging in size from 100 to 5000 nm, including airborne tiny droplets.[41,42]

NIOSH recognized different types of respirators are most popularly known as FFP respirators. As per the shape, it may be (i) cup model type or (ii) fold model type or (iii) linear model type.[43] (Fig. 5).

- **NIOSH respirators filter masks:**

The NIOSH respirators screening masks are respiratory guarding equipment manufactured to conform a remarkably close facial clothing and very productive filtration of airborne components.

4.1. Types of NIOSH respirator filter masks

Based on filtering particles and efficiency of resistance to oil, NIOSH respirator filter are of three types: N, P, R.

Depend on particle filtering efficiency *N*-type respirators are of three categories- N95, N99, and N100. N95 respirators are generally used in healthcare vicinities and are a subset of N95 Filtering Facepiece Respirators (FFRs), often cited as N95s.[37] The N95 designation means that under experimental circumstances which are approved by the United States CDC and National Institute for Occupational Safety and Health, the respirator hurdles at least 95 % of strong and watery aerosol trial components. The most incredibly used is N95 which is called electrets filters, which has a filtration of 95 % of aerosols N90/N95 face mask is one of nine NIOSH certified particle respirators. Obtaining an N95 mask as an example, “N” means not resistant to oil. “95” means that the particle concentration in the mask is 95 % poorer than that outside the mask when disclosure to a specified number of special test particles. 95 % is not the normal filter rate, but the slightest value. N95 is not a certain product name. As long as the product meets the N95 criterion and upholds NIOSH review, it can be called “N95 mask”. The degree of safety is N95, which means that under the test circumstances stipulated in NIOSH standard, the filtering efficacy of the filter substance of the mask for non-oily particles (such as acid mist, dust, paint mist, microorganism, etc.) attains 95 %. Nevertheless, various companies manufacture several N95 and its effectiveness relies on the size of penetrating particles. It has 4 layers-inner, filter, support and layer mask filter layer from outside to inside with a ventilator fan to permit reinforce breathing. N95 are of two categories- The standard N95 and surgical N95, which is more worthwhile.[40] (Fig. 6).

Some other variants to the N95 mask are also available like N90, valved N90, valved N95, KN90 and KN95. In the case of the N90 mask, the number 90 signifies the effectiveness of the mask to filter out 2.5 p. m. dust particles. While KN90 respirators with valves are more favorable for industries of non-ferrous metal processing, food processing, metallurgy, construction works and all additional oil and non-oil particles pollutants such as dust particles, smoke fog. KN90 can apprehend more than 90 % of particles. Although it is not as beneficial as KN90 in the case of particle conservation. KN90 is a promising choice to travel in a mist for a short time.[11,40]

There are a variety of respirators on the market that match the same design criteria as N95s, and they are approved as KN95s in China. These respirators are fundamentally the same. The KN95 filters out at least 95% of particles down to 0.3 μm (Fig. 7). To be more useful, these respirators also must develop a seal around the nose and face when worn. Genuine N95s will have NIOSH written in block letters on the respirator, including testing and certification numbers, as well as the identification, N95. This can help recognize the fake masks on the markets.[40]

The usage of valves in face masks such as the valved N90 and N95 are stated as not prohibiting the virus from escaping out of the mask. The valve is almost a ‘one-way valve’ that only insures the person wearing it and does not purify the aerosols coming out. So, an asymptomatic person with coronavirus can scatter the infection to people when the valve discharges the unfiltered exhaled air in the sudden environment. Therefore, in a sealed area, people around the carrier have an increased threat of conceivable disclosure to the Covid-19. Asymptomatic transmission disseminates the infection to another person. On the other hand, a mask without a valve will not permit the virus to circulate.[40]

The R and P masks have friction to oils but the high-performance 100 refers to the lowest percentage of factors screened under trial conditions. Both R-type (Resistant to oil) and *P*-type (Strongly Resistant to oil/Oil Proof) of respirators differentiate into three types, viz., R95, R99, R100, and P95, P99, P100 respectively. Their filtering power is at least 99 % and 99.7 % respectively.[42] The P100 respirator has a filtration of 99.7 %. Studies were performed to find and distinguish the efficiency of N95 and P100 before and post-exercise. The permeability values were more or less the same with both before exercise. But, the outline after exercise demonstrated change, having the benefit of using P100 masks.[40]

(ii) Full-length face shield:

It is made out of elastic headbands and a clear polycarbonate shield that runs across the face (Fig. 8). It prohibits the wearer from splashes of coughing and other liquid droplets. It had the advantage of being light and cost-effective. It is primarily used in a clinical area.[40]

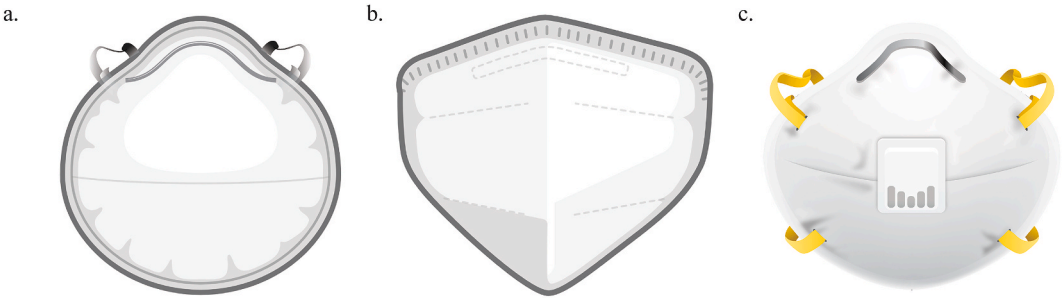

Fig. 5. Diagram shows the different shaped Filtering Face-Piece Respirators **a.** Cup Model Type **b.** Fold Model Type **c.** Liner Model Type (Created with BioRender.com).

Fig. 6. Diagram shows the N95 mask and its layering pattern: **a.** N95 mask without respirator **b.** N95 mask with respirator **c.** Different layering pattern of N95 mask without respirator.

Full-length face shields come through different structures, but all deliver a plastic boundary for the face to protect against droplets and virus particles. The faceguard coverings will ensure that it reaches the chin and that there is no gap between the forehead and the face-shield guard. The laboratory study showed that the face guard was capable of inhibiting 96 % viral particles when worn by health care personnel within 18 inches of cough.[44] Yet social distancing, wearing masks must be important for avoiding viral respiratory diseases.[22,45]

(iii) Self-contained breathing apparatus (SCBA)

It comprises a facepiece that is connected to an allowance of liquid air or liquid oxygen. A hose and a regulator are used to keep the SCBA in place (Fig. 9). It primarily gives protect against airborne pollutants, making it easy for those working in a smoky environment. It is employed as personal firefighting cautionary. It has the disadvantage of being hefty, which limits the user's mobility in the workplace.[40]

Table 1 shows the many types of masks, their materials, characteristics, and purposes, as well as their percentage of effectiveness in protecting against the Coronavirus.

4.2. *Mechanistic significance of wearing a mask*

Over the last several months, the use of facemasks has been identified as one of the most important and cost-effective mitigating strategies for delaying COVID-19 transmission. In addition to government and public health officials' advice and mandates for mask use, a growing number of scientific studies have demonstrated the effectiveness of masks and universal masking.

Face Masks and different PPE items serve as a physical obstacle to respiratory droplets. An in vitro model with basis and receiver figures was developed to test the impact of the mask on filtering away radio-labelled aerosol. Masking at the source mannequin was invariably more beneficial at reducing radio-labelled aerosols entering the receiver mannequin, whereas the only practical setup where the receiver mannequin could be equally adequately conserved was if the receiver mannequin wore an N95 mask enclosed with Vaseline.[47] Hence, masks can work as a physical obstacle and seem to be more significant when worn by an infected person.

The use of a mask by the infected person helps in reducing virus transmission. The surgical mask was examined for its potentiality to hinder the discharge of numerous viruses by investigating the quantity

Fig. 7. Diagram shows the K95 mask.

of virus existing in the exhaled breath of the infected person. The researchers were able to obtain the particles distinguished by size (>or <5 μm). With the mask on, a critical decline in coronaviruses in both smaller and bigger particles was investigated. The mask reduced the number of influenza viruses identified in bigger particles, but not in smaller particles. After wearing a mask, no coronavirus was found in any of the 11 patients, although influenza was found in the respiratory particles of one of them.[48] This means that surgical face masks can reduce coronavirus and influenza transmission from an infected person. Participants were encouraged to cough for influenza trial, and no influenza could be detected by reverse transcriptase-polymerase chain reaction (RT-PCR) for 9 infected patients using both N95 and surgical masks.[49] When the exhaled influenza virus was divided into amounts based on size, surgical masks were found to be highly effective at eliminating influenza from the large coarse portion (≥5 μm).[50] Individuals who come into contact with an infected person can benefit from wearing face masks. During the SARS outbreak in Hong Kong, hospital faculties were asked about the preventative measures they took and this data was linked to whether or not they were infected. Wearing face masks was determined to be the most effective preventative measure in reducing the risk of infection, and those who wore surgical masks or N95 masks were not among the 11 infected employees. Nonetheless, two groups of people who wore paper masks became sick, implying that the types of masks were also important.[51] A study distinguished the effectiveness of surgical and N95 masks against viral respiratory infections in healthcare employees. When healthcare workers used surgical masks or N95 masks, there was no significant difference in influenza infection outcomes, implying that both types of medical masks can protect equally.[52] A meta-analysis was conducted on clinical surveys to investigate the protective impact of masks. Wearing a face mask protect persons against influenza-like illness, illustrating a risk ratio of 0.34, with a 95 % confidence duration between 0.14 and 0.82. According to the study, there was a minimal difference in protection between N95 masks and surgical masks, with a hazard ratio of 0.84 and a 95 % confidence duration of 0.36–1.99, indicating no significant difference in risk.[53] A study conducted by Eikenberry et al. suggested that the widespread use of masks by the general public can significantly reduce population transmission rates and death tolls.[54] Hence, the study finds that the widespread use of face masks has the potential to significantly minimise community transmission and the threat of a COVID-19 pandemic. These data can be used to support and inform public health communications campaigns and undertakings involving the use of masks to help limit the spread of COVID-19, as well as to examine how policies and practices interact around the world.

4.3. *Caring methods of non-medical masks*

Masks should be worn by only one person and should not be shared. When masks become wet or dirty, they should be changed immediately; a patchy mask should not be worn for an extended amount of time. While removing the mask avoid touching the front side of the mask and do not touch any other part of the face after removing. Abandon the mask or keep it in a sealable pouch until it is cleaned and washed again. Afterwards, instantly practice hand hygiene. Non-medical masks should be washed frequently and handled carefully.

Do not use the mask, if the fabrics look notably worn out. The highest permissible washing temperature for clothing textiles used to make masks should be checked. Clean it in with soap or detergent in warm, hot water (60 °C). Non-woven polypropylene (PP) spun-bond perhaps washed at high temperatures, up to 125 °C.[55] Naturalistic fibres may fend with high-temperature ironing and washes. Cleanup the mask exquisitely but avoid too much abrasion, extending or rubbing. The summation of cotton and non-woven PP spun bond can undergo high temperatures, masks formed of these compounds perhaps fumigated or steamed.

When warm water is not obtainable, wash the mask with detergent or soap at room temperature water, pursued by either (i) steaming mask for 1 min or, (ii) drench face cover in 0.1 % chlorine for 1 min. Rinse the mask properly with room temperature water to avoid any toxic chlorine accessorial.

Though the cloth masks may be created in huge quantities in a short amount of time, they can be reused after being decontaminated using a variety of methods, the most effective of which is washing in hot water with soap. Other methods or products, to get contaminant free cloth masks, are the use of bleach, isopropyl alcohol, or hydrogen peroxide;

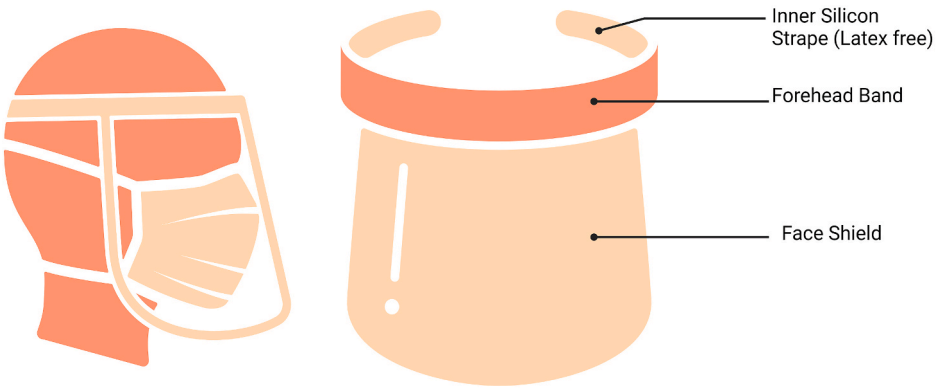

Fig. 8. Diagram shows the full-length face shield and its layering pattern (Created with BioRender.com).

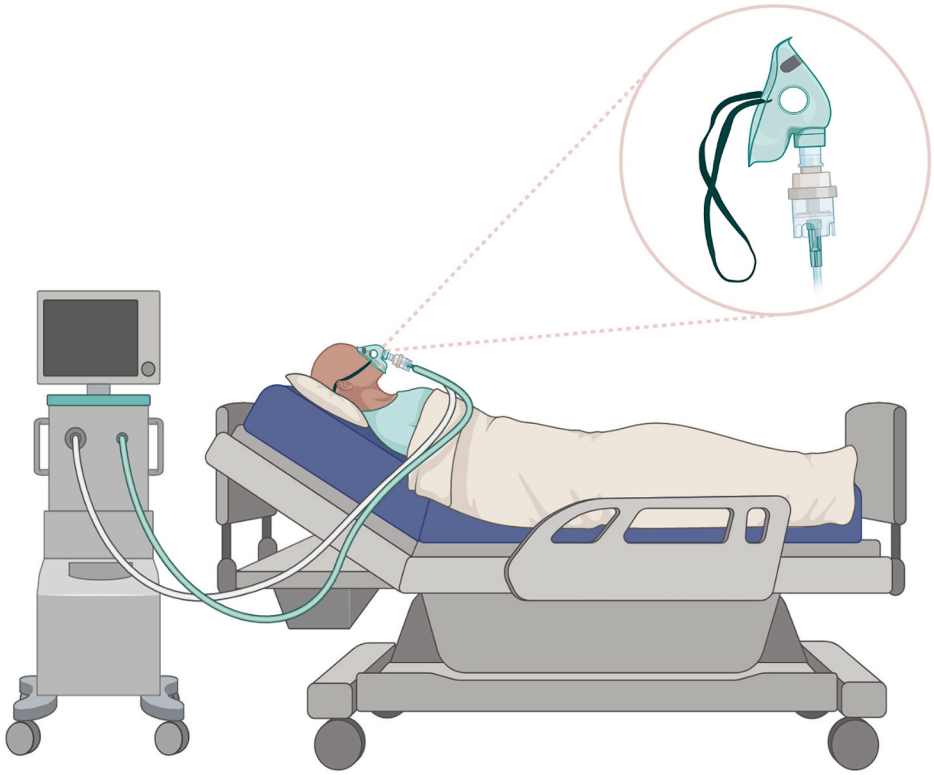

Fig. 9. Diagram shows the Self-Contained Breathing Apparatus (Created with BioRender.com).

Table 1
A detailed summary of the different types of masks and their effectiveness in protecting against the SARS-CoV-2 virus.

Type of mask	Materials used	Description	Efficiency in percentage	Purpose	Respiratory filter	Reusable	Reference
Cloth Face Mask	Common textiles, usually cotton	The most protective cloth face masks require at least three layers with a hydrophilic inner layer (e.g. cotton) to consume moisture from the wearer's breathing and hydrophobic outer layers (e.g. polyester).	Zero % efficient at 0.3 μm	Dust Party leader, virus and bacteria	No	Reusable	40,46
Surgical Face Mask	Non-woven fabric	This mask has been certified by the Food and drug administration. This category of mask protects the mouth, nose, eye, cheeks and forehead. It is mostly referred to the Medical professional dealing with operations drive through COVID-19 patients.	60%–80 % filtration of particles as small as 0.3 μm	Virus, bacteria, pollen and dust particles	No	Disposable	40,46
N95 Face Mask	Fine mesh of synthetic polymer fibres, specifically a non-woven polypropylene fabric.	This mask is mostly recommended by health care workers and first medical responders dealing with COVID-19 patients.	95 % efficient of particles sized 0.1–0.3 μm	Virus, bacteria, pollens, liquid like sprays against non-oil particles	Yes	Reusable	12,40,46
FFPR (P100) Face Mask	Fine mesh of synthetic polymer fibres, specifically a non-woven polypropylene fabric.	Unlike the surgical mask, this type of mask is mostly used to stop the spread of airborne diseases and also is dispensable.	99.97 % efficient of particles sized 0.1–0.3 μm	Virus, bacteria, dust particles.	Yes	Reusable	12,40,46
KN95 Face Mask	Non-woven fabric, often made from polypropylene.	They are composed of four layers: outer, filter, cotton and inner layers.	80%–95 % efficient of particles down to 0.3 μm	Virus, bacteria, pollens, liquid-like sprays against non-oil particles	Yes	Reusable	40
Face shield	Flimsier	This mask is made of flimsier, which cover the entire face from the forehead to chin and is secure with a headband cushioned.	Effective with perhaps surgical or N95 masks worn.	Liquid like most sprays.	No	Reusable	40,46
SCBA	A back-plate that holds the cylinder and reduces the air from high pressure (200–300 bar) to medium pressure (5–11 bar) and, in turn, supplies a face mask.	Mostly worn by firefighters to ease fresh breath when in contact with hazardous environments.	99 % of knotter than 0.3 μm.	Emergency conditions, viruses, bacteria, smoke particles, and non-oil particles.	Yes	Reusable	40,46

autoclaving or microwaving; and the application of ultraviolet radiation or dry heat.[56] Unlike disposable medical masks and respirators, the material of cloth masks is unlikely to degrade from standard decontamination procedures. Hospitals, on the other hand, will be burdened with the additional task of washing and decontaminating worn masks. If healthcare personnel do decontamination on their own, they may not wash masks frequently enough, putting themselves at risk of infection.[12]

If you wear a none medical mask, do not touch it unnecessarily. Masks of any type are not comfortable and can cause users to touch their faces often to adjust the mask. This can be complicated, as that is specifically how the virus would enter your body.

Cloth masks should be washed after each use, or sooner if they appear soiled. Follow any particular instructions provided by the manufacturer. Use the warmest washer and dryer settings that are safe for the fabric. If you don't have access to a dryer or washer, you can wash a face mask in bleach and air dry it. To prepare the solution, add 4 tablespoons of bleach in 1 L of normal water, then saturate the mask in that solution for 4–5 min. After saturation, wash the mask thoroughly with normal water and let it air dry completely in presence of sunlight.[57]

In a nutshell, one must follow the following protocols to maintain the hygiene of the masks:

A. Store wet or dirty masks in a plastic bag: If your mask is filthy or wet from saliva, sweat, make-up, or different liquids or entities, keep it in an impenetrable plastic bag until you can clean it. To avoid mould growth, wash contaminated or wet masks as soon as possible. Damp masks are less useful than dry masks since they are difficult to breathe through.[58,59]

B. Masks that aren't damp or soiled should be stored in a clean paper bag: You can save your mask for later use by temporarily storing it. After touching a used mask, properly dispose of it and wash your hands. To keep it clean between uses, store it in a dry, breathable bag (such as a paper or mesh fabric bag). Keep the same side of your mask facing out while reusing it. If you need to remove your mask to drink or eat outside of the house, place it in a safe place, such as your pouch, handbag, or paper bag. After removing your mask, make sure to rinse or sanitize your hands. Replace the mask with the same side facing out after eating. After re-applying your mask, make sure to rinse or sterilise both hands once again.[58,59]

C. Wash your mask: Wash your cotton mask whenever it gets soiled, or at least once a day. If you're using a disposable face mask, throw it away after one use.[58]

(i) Using a washing machine: Include your mask in your regular laundry. Use ordinary laundry detergent and the appropriate settings for the fabric, as directed on label.[58]
(ii) By hand: Rinse your mask with soap or laundry detergent and water. To get rid of any soap or detergent, wash it fully with clean water.[58]

D. Dry your mask: Use a warm or hot drier to completely dry your mask. Allow your mask to dry completely in direct sunlight. If you can't dangle it in the sunshine, hang it or spread it out flat to dry.[58]

WHO is cooperating with research and development partners and the scientific community involved in fabric design and textile engineering to simplify a fine knowledge of the efficiency and utility of non-medical masks. WHO impulse countries that have emanated recommendations on the usage of both non-medical and medical masks by having people in community settings to propulsion research on this significant issue. Such research is urgent to look at if SARS-CoV-2 particles may be eradicated by non-medical masks of impoverished integrity worn through an individual with syndromes of COVID-19 if that individual is sneezing, speaking, or coughing. Research is also required on non-medical mask use by children and several medically difficult individuals and settings as above mentioned.

4.4. Categories of individuals who should wear medical masks against the expanse of COVID-19

Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus, which was recently found. The mask is used in many ways depending on the type of person.[60] These divisions are described properly below:

4.5. Hospital

4.5.1. Monitoring area

During interacting with victims, all medical crews such as nurses and the paramedical team would use disposable triple-layer surgical masks.

4.5.2. Isolation department

In the isolation cabins, all victims should be maintained that they must wear a disposable triple-layer surgical mask. Nursing and medical staff included in clinical caring in isolation faculties would need a triple-layer surgical mask, including extra Personal Protective Equipment (PPE). Nevertheless, if the staff is related to any aerosol-generating protocols like intubation, suction and nebulization then they must use the N95 respirator. Likewise, if the medical crew requisite to obtain clinical specimens from victims, then they must use N95 respirators.[60]

4.5.3. Critical care faculty

While nursing and medical staff are implicated in crucial care in the Intensive Care Unit (ICU) then they must utilize N95 Respirators.

4.5.4. In laboratory room

In laboratories, all staff are working and examining clinical samples related to pathogens like Influenza then they should use N95 Respirators.

4.5.5. Mortuary

Personnel involved in dealing with dead bodies of verified patients of seasonal influenza should utilize a triple-layer surgical mask, along with other infection measure procedures.

4.5.6. Ambulance staff

The motorist of the ambulance appointed for shifting patients with Influenza should utilize a triple-layer medical mask. The paramedics in the patient bedroom should borrow a triple-layer surgical mask and though the performance of any aerosol-generating procedures are contemplated (suction, oxygen administration by nasal catheter, intubation, nebulization, etc.) Likewise, in these situations, an N95 respirator should be worn at all times.[60]

4.6. Health employees in community setting

4.6.1. Doctors

Doctors treating Influenza-Like Illness (ILI) in general practice, as well as other health care workers working with them, should utilize a triple-layer surgical mask at the screening centre.[60]

4.6.2. Healthcare workers

Health workers involved in community surveillance contact tracing and health monitoring of cases at home or under home quarantine should use a triple-layer surgical mask.[22]

4.6.3. Security personnel

When operating in an infected/potentially infected location, such as an influenza ward in a screening centre or a hospital, security staff should wear a triple-layer surgical mask.[22]

The specifics of individuals who should use a medical mask or other respirators during certain activities are listed in Table 2.

4.7. Guidelines for using the mask for the general public

Many countries recommend fabric masks or homemade masks for the common public to prevent the spread of pandemic disease.

4.7.1. Public settings

Masks should be worn in public locations, such as grocery shops, public meetings, at work, closed locations, along with schools, colleges, temples, mosques, churches, etc. In the above criteria, people should use a non-medical mask such as a cloth mask.[60]

4.7.2. During transport

Non-medical masks should be worn on public transit and in specific work scenarios that bring the person in close contact or potentially close contact with others, such as cashiers, social workers, and servers, and medical masks should be used only when necessary.[60]

4.7.3. Senior citizens

When people's age is above≥60, then they should use medical masks. However, N95 respirator masks can also be used for protection.[60]

4.7.4. Other people

People with underlying comorbidities, such as cardiovascular illness or chronic lung disease, diabetes mellitus, cancer, cerebrovascular disease, immunosuppression must use a medical mask.[22,60]

4.7.5. Symptomatic public

Persons with any syndromes suggestive of COVID-19 and close family contacts of such suspect/confirmed cases undergoing home care should also use a triple-layered medical mask.[22,60]

To prevent the coronavirus outbreak, the mask should be worn by[62,63]:

1. People who are 2 years of age and senior citizens.
2. Whenever you are in social background.
3. Each time when you are travelling on a bus, plane, train, or different kinds of public vehicle. Wear masks also in-vehicle hubs such as stations and airports.
4. At the time, when you are around folks who do not occupy with you, constituting inside your house or inside somebody else's residence.
5. Inside your house if somebody you stay with is sick with signs of COVID-19 or has tested positive for COVID-19.

Although, CDC acknowledges there are certain instances when wearing a face mask may not be conceivable always. In these instances, evaluate alternatives and adaptations.

The subsequent types of people can be exempted from the requirement to wear a face mask[62,63]:

1. A kid under the age of 2 years;
2. A person with a disability who cannot, or cannot safely, use a face mask for reasons related to their condition.[62,63]
3. Certain groups of people who may find it difficult to wear a mask: Relevant and consistent use of masks may be struggling for some children and communities of any age with specific disabilities, along with the people who have high sensitiveness to substances on their faces, trouble realizing why wearing a face mask is defensive (such as people with an intellectual disability), and those who have issues regulating their behavior in social situations.[62,63]
4. Those caring for youngsters and those with specific disabilities who may need assistance with wearing masks. In that scenario, they should seek advice from their healthcare providers concerning the person they're caring for who is wearing a mask. If they are unable to wear a mask, speak with their healthcare providers about other ways to reduce transmission risk and ensure proper mask fit and size. They should discard their mask before napping, sleeping, when they may

Table 2
Categories of individuals who should wear medical or other respiratory masks.

Setting	Categories on individuals	Types of masks	Activity	Reference
Screening	Health Care Workers (HCWs).	Medical mask.	Preliminary contact not comprising direct contact.	46,61
	Patients with signs suggestive of COVID-19.	Medical mask.	Any	34,46,61
	Patients without symptoms suggestive of COVID-19.	If the prevalence of COVID-19 is high, utilize a medical mask. But when the condition is in control, cloth face coverings/ masks may be used.	Any	46,61
Patient room	Health Care Workers (HCWs)	Medical mask	1. Providing direct care (no aerosol-generating procedures) 2. Direct care (aerosol-generating methods are repeatedly in place)	46,61
	Cleaners	FFP2/N95 respirators or medical mask if available	When they are entering the patient's room for cleaning or in general when they cleaning contaminated stuff.	46,61
Administrative rooms	All staff, along with HCWs.	If they are not in direct contact with patients suffering from COVID-19 they can Maintain physical distance (if that is not possible) then they should wear an N95 or cloth face masks.	Administrative duties that do not comprise contact with COVID-19 patients.	46,61
Laboratory	Lab technician	N95/FFP2 respirators	Manipulation of respiratory specimens of supposed COVID-19 patients.	34,46,61
Waiting compartment	Patients with signs suggestive of COVID-19	Medical mask	Any	46,61
	Patients without signs	Mask is not required if physical	Any	46,61

(continued on next page)

Table 2 (*continued*)

Setting	Categories on individuals	Types of masks	Activity	Reference
	suggestive of COVID-19	distance is maintained.		
Consultation room	Health Care Workers (HCWs)	Medical Mask	Physical inspection	46,61
	Patients	Medical Mask	Any	46,61
	Cleaners	Medical Mask	After and between consultations with patients with respiratory signs	46,61
Locations of transit where patients are not permitted	All staff, along with Health Care Workers	Maintain physical expanse (if not possible, cloth face masks can be utilized).	Any kind of activity that does not include physical contact with COVID-19 patients	46,61
Home	Patients with signs suggestive of COVID-19	Medical mask	Any	46,61
	Caregivers	Medical mask	Entering patients' rooms or providing direct care	46,61
	Health Care Workers	Medical mask	Entering patients' room or providing direct care	46,61
Community Settings	Anyone	Maintain physical distance (N95, FFP respirators and cloth face masks can be used).	Outdoors	46,61
		Maintain physical distance. No mask required	Indoors	46,61

fall asleep (such as in a stroller or car seat), and in conditions when continuous maintenance is not feasible.[62]

But consider prioritizing wearing a mask at least in public locations and when around people who don't live in your home, especially when indoors. Masks may not be essential when you and the person you are looking after is away from each other, or with other people who live in the same home. Though some neighborhoods may have mask requirements while out in public and these requirements should always be obeyed.[62]

People who are deaf or having difficulty in hearing, and those who will interact with people who have difficulty in the hearing should consider wearing a cloth mask with a clear panel. If you are unable to get a clear mask, consider utilizing written communication, closed featuring, or reducing background noise to make communication reasonable while wearing a mask that blocks lips.

People with specific underlying medical conditions like having any respiratory problem or asthma can also wear a mask, but they should first take advice from their doctor.[62]

If a person works in an area where masks could increase the risk of heat-related disease or cause safety issues (such as straps getting stuck in machinery), they should consult with a healthcare expert and occupational safety to determine whether the mask is appropriate or not.[62]

4.8. Guidelines about utilizing masks in pandemic COVID-19

Masks may be used for safety for healthy persons (when worn to defend themselves when in contact with an infected person) or for source supervision, according to the WHO's revised guidelines (worn through an infected person to obstruct onward transmission).[64]

The current protocols over face masks usage have deviated from the present guidelines provided by WHO. It was argued that there was insufficient evidence to suggest that healthy persons should wear face masks and that surgical face masks should only be worn by those who were sick or caring for patients.[64]

4.9. Guidance on the use of masks in health care settings

In the context of situations with acquainted or suspected society transmission or severe explosions of COVID-19, WHO provides the following guidance[64,65]:

- Health employees, along with community health employees and caretakers, who are employed in clinical sectors should constantly wear a medical mask at the time of their periodic activities throughout the whole shift; apart from when drinking and eating or altering their mask to look after a patient who compels droplet precautions for other purposes.[64,65]
- According to the WHO statement, it is incredibly significant to obtain the constant usage of masks in probable massive transmission hazard areas along with triage, household physician, GP practices, emergency areas, outpatient divisions, cancer, transplant departments, long-period health crisis, COVID-19 determined departments and residential faculties.[64]
- Throughout the whole shift, at the time of utilizing medical masks, health employees should be assured that the medical mask is altered when damp, stained, or ruined; the medical mask is never contacted to adjust it or replaced from the face for any intention; the medical mask (as well as different personal protective equipment) is omitted and altered after taking care for any patient on droplet precautions for other pathogens.[65]
- Faculty members who do not work in the medical field are not required to wear a mask throughout their daily activities.
- Masks should never be shared among health professionals and should be suitably eliminated whenever discarded and never reused.
- A particulate respirator is as defensive as a US National Institute for Occupational Safety and Health-approved N99, N95, US FDA surgical N95, European Union standard FFP2 or FFP3, or equivalent, should be worn in environments for COVID-19 victims where AGPs are conducted. In these environments, the constant usage of masks by health professionals throughout their whole shift is highly recommended.[64,65]
- To be completely beneficial, the consecutive wearing of a face mask among health employees, throughout their exhaustive shift, should be executed along with maintaining constant hand hygiene and corporal distancing between health staff in disseminated and congested areas where mask usage can be unfeasible such as restaurants, clothing spaces, etc.

The probable risks and hazards should be gingerly taken into report when acquiring this strategy of targeted continuous usage of medical mask, which includes:

- Self-contamination owing to the manipulation of the face mask by filthy hands.[30,31]
- Probable self-contamination can appear where medical masks are not altered when damp, contaminated or destroyed.

- Conceivable advancement of facial skin injuries, stinging dermatitis, or deepening acne, when utilized for several hours.
- Masks perhaps uneasy to wear.
- Erroneous understanding of protection, heading to potentially minor allegiance to well-acknowledged preventative criteria certain as hand hygiene and corporal distancing.
- The hazard of droplet transmission and splashing to the eyes, whether mask-wearing is not integrated with eye safety.
- Inconvenience for or complication wearing masks by particular susceptible communities certain as those including mental health illnesses, the deaf, developmental disabilities, rough of hearing society, and children.
- A complication of wearing them in warm and moist atmospheres.[66]

4.10. Guidance on the use of masks for the general public

WHO approves that individuals with any symptoms suggestive of COVID-19 should follow these guidelines [64,65]:

- Wearing a medical mask, self-sequester, and look for the medical recommendation as early as they begin to feel sick with possible symptoms of COVID-19, even if symptoms are mild. Symptoms include cough, fever, tiredness, loss of enthusiasm, muscle irritation, and shortness of breath. Other non-particular signs include bruise throat, diarrhoea, nasal congestion, headache, vomiting, nausea, losing of taste and odour foregoing the beginning of respiratory signs have further been reported.[67,68]
- Senior people and immunosuppressed sufferers may have anomalous symptoms such as exhaustion, lowered alertness, lessened mobility, diarrhoea, delirium, loss of hunger, and lack of fever.[69–71] It is significant to report these initial symptoms for few people infected with COVID-19 may be very mild and indefinite.
- One must follow instructions on how to wear, take off, and dispose of face masks and maintain hand hygiene.[22]
- All additional conditions, particularly in respiratory hygiene, thorough hand washing, and maintaining a social distance of at least 1 m from other people, are required.[72]

In the circumstances of the COVID-19 pandemic, it is instructed that all individuals, nonetheless of whether they are utilizing face masks or not, should:

- Avoid mass gatherings.
- The physical distance of at least 1 m from another person should be followed, especially from the person with respiratory symptoms like gasping, coughing or sneezing.
- Practice hand hygiene repeatedly uses an alcohol-based hand sanitiser or soap and water.
- Use respiratory hygiene by covering the nose and mouth with a bent elbow or tissue paper when gasping or sneezing, after usage, discard the tissue instantly, and sanitize your hands with a sanitiser or soap and water.
- One should refrain from constant touching of eyes, nose and mouth.

4.11. Guidance on the use of medical masks for the care of COVID-19 patients at home

WHO delivers advice on how to look after patients with suspected COVID-19 at the household when looking after in a medical facility or other residential setting is not possible. Home care may be considered when isolation in non-traditional settings is unavailable or unsafe. Specific guidance for the use of medical mask at-home care is:[73]

- Always wear a mask and at least once a day changes the mask and use the fresh and dry mask.
- Individuals who cannot tolerate a medical mask must maintain respiratory hygiene and practice hand hygiene frequently.
- Always wear a mask when present in the same room as the infected person.

4.12. Probable advantages of using masks[22,73]

The potential benefits of the usage of masks by a healthy person:

- It helps in reducing the risk of exposure to infection from infected individuals before they develop any symptoms.
- Minimized probable stigmatization of people wearing masks to prohibit infecting others or of people looking after the COVID-19 patients in non-clinical environments.
- Make people realize that they can play a part in contributing to stopping the spread of the virus.
- Making people be responsible citizens by practising hand hygiene, not touching nose and mouth.
- It also provides social and economic privileges. Amidst the worldwide deficiency of PPE and surgical masks, urging the public to generate their cloth masks can emphasize personal business and public integration. Additionally, the output of non-medical masks may extend an origin of earnings for those who elect to develop masks among their societies.
- Fabric masks can further be a kind of cultural representation, motivating the public to an acknowledgement of safety regulations in general. The protected reuse of fabric masks will furthermore lessen waste, prices, and contribute to sustainability.

4.13. Mask Management[73]

For several types of masks, relevant usage and dumping are necessary to assure that they are beneficial and to ignore any upgrade in transmission.

WHO (World Health Organization) recommends the subsequent guidance on the appropriate usage of masks, derived from best procedures in health care settings[22,73]:

- Before wearing the mask, always ensure hand hygiene.
- Avoid contacting the mask while wearing it.
- Wear the mask cautiously assuring it coats the nose or mouth, adjust to the nose, and wrap it securely to reduce any gaps between the face and the mask.
- Discard the mask using a reasonable technique.
- While removing the mask, do not touch the front part of the mask and remove it from the back.
- After disposal or a used mask is inadvertently touched, immediately clean hands with an alcohol-based sanitiser, or soap and water.
- Don't reuse again single-used masks.
- Replace the mask as early as it becomes damp with a new clean, dry mask.
- Abandon the single-use masks after each use and dispose of them carefully.

5. Conclusion

Covid-19 has created a huge impact on economic, educational, psychological, and people's livelihood worldwide. Regulating SARS-CoV-2 transmission at the source by a face mask is a well-established strategy. Medical mask and N95 mask shortages have become major problems all over the world. Homemade cloth or fabric masks, however, were highly recommended by researchers as a way to reduce COVID-19 transmission. As per reports, there are no proven vaccines or specific treatments available which show cent percent efficacy to prevent this pandemic yet. Though the work has been going on vaccination by International research organizations and they developed several vaccines

to date but nothing provides the hundred percent effectiveness against COVID-19. Until then, it is recommended that every individual should wear face masks in community places and maintain other protective measures. Everyone as a responsible citizen must follow the guidelines and instructions recommended by WHO and the Government to combat the pandemic situation.

The general public can use cloth masks especially cloth mask 1 as an alternative to medical and surgical masks in case they are not available. Till now, the three-layered cloth mask 1 provides the best filtering technique. Besides this people can also use the KN95 mask as an alternative to the N95 mask because it also provides similar effectiveness as N95 masks.

It is recommended for people to wear a mask whenever possible to prevent themselves from the coronavirus outbreak.

Author contributions

Conceptualization, J.S.; resources, S.D., A.D. and S.D.; writing—draft preparation, S.D., A.D., S.D., and S.S.; writing—review and editing, J.S. and S.S.; image preparation, P.C. and J.S.; visualization, J.S.; supervision, J.S.; All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

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Mucormycosis: A new threat to Coronavirus disease 2019 with special emphasis on India

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ABSTRACT

The main reason for the growth of mucormycosis in people with Coronavirus disease-2019 (COVID-19) is mainly produced by *Rhizopus* spp. The infective mechanisms and issues recognized in *Rhizopus* spp. are the cell wall, germination proteins, and enzymes assisted to iron sequestration, CotH protein, and positive regulation of the GRP78 cell receptor. Mucormycosis is mainly caused by the *Rhizopus* spp. such as *R. oryzae, R. microsporus, R. arrhizus, R. homothallicus*, etc. that are gifted to numerous host defense mechanisms and attribute to the endothelium via specific receptors, GRP78 simplifying their endocytosis and angio-invasion. Factors such as hyperglycemia, elevated iron concentrations, and ketoacidosis have been shown to contribute to the pathogenesis in the tentative situation. The analytical data of 'black fungus disease' or 'mucormycosis', specify India reported for about 42.3% of published cases, followed by the USA about 16.9%, Iraq, Bangladesh, Iran, Paraguay, and 1 case each from Brazil, Mexico, Italy, UK, China, France, Uruguay, Turkey, and Austria. The COVID-19 infection is maybe a predisposing factor for mucormycosis and is related to a high mortality rate. Early recognition and restriction of hyperglycemia, liposomal amphotericin B, and surgical debridement are the bases in the successful managing of mucormycosis.

1. Introduction

Mucormycosis is an uncommon angio obtrusive disease principally perceived in immunocompromised patients which happens because of the growth of mucorales.[1] The term 'Mucormycosis' was instituted by an American pathologist R. D. Baker and it can likewise be called Zygomycosis. Mucormycotina falls under the normal saprobes which are found in bad organic matter or soil. Infections are designated by instantaneous progression.[2] The Mucorales are not demanding creatures, they develop at temperature ranges between 25 °C and 55 °C.[1] Being ubiquitous organisms, Mucorales are dominant in commencing and accelerating the decay of organic materials. Since openness to spores of these growths is unavoidable, the uncommonness of the diseases is harmful and is a validation of an extremely basic inclination.[3]

The initially announced instance of mucormycosis traces back to 1885 when the German pathologist Paltauf depicted the primary case as Mycosis Mucorina.[4] The pace of mucormycosis expanded mostly in immunocompromised individuals subsequently in the 1980s–1990s.[2]

Different types of mucormycosis that can be associated with COVID-19 infection are, rhino-cerebral mucormycosis, pulmonary mucormycosis, gastrointestinal mucormycosis, cutaneous mucormycosis, and miscellaneous. For the region of the head and neck, mucormycosis can be assorted into isolated nasal, rhino-orbital, or rhino-orbital-cerebral mucormycosis. In the case of sino-orbital mucormycosis, the mold mainly enters via the respiratory tract and is containing the nose and sinuses, into the orbital and intracranial structures with the possibility of further progression.[5,6] Pulmonary mucormycosis is a lethal aggressive fungal infection. It typically infects immunocompromised patients. Transbronchial biopsies and Bronchial alveolar lavage (BAL) are usually explained as non-septated hyphae in the case of pulmonary mucormycosis.[7] Mucormycosis in the gastrointestinal (GI) tract occurs due to the ingestion of the spores of the fungus. It is rarely reported in the COVID-19 patient.[8] Patients with persistent skin maceration or skin barrier disruptions (catheter insertion, trauma, injections, burn) are

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suitable for increasing the risk of cutaneous mucormycosis.[9] The fungus can invade into adjacent fat, fascia, muscle, and even bone, while hematogenous spread with secondary vascular invasion is fewer common.[10,11] However, hematogenous dissemination with cutaneous mucormycosis has high fatality rates.[12]

From the perspective of disease to the immunocompromised people, mucormycosis likewise create a high danger for the patient determined to have serious COVID-19 pneumonia. This happens because of the hospitalized status, previous comorbidities, and treatment regimens comprising of steroids and generally anti-toxins.[13,14] The predominance of mucormycosis in India is approximately 0.14 cases per 1000 populace, about multiple times the pervasiveness in different countries.[15] COVID-19 contamination has been related to parasitic diseases.[16] Globally, the most well-known danger factor related to mucormycosis is diabetes mellitus. In the prevalence of the COVID-19 pandemic, it is believed that this drop in resistance could be set off to these instances of mucormycosis.[17]

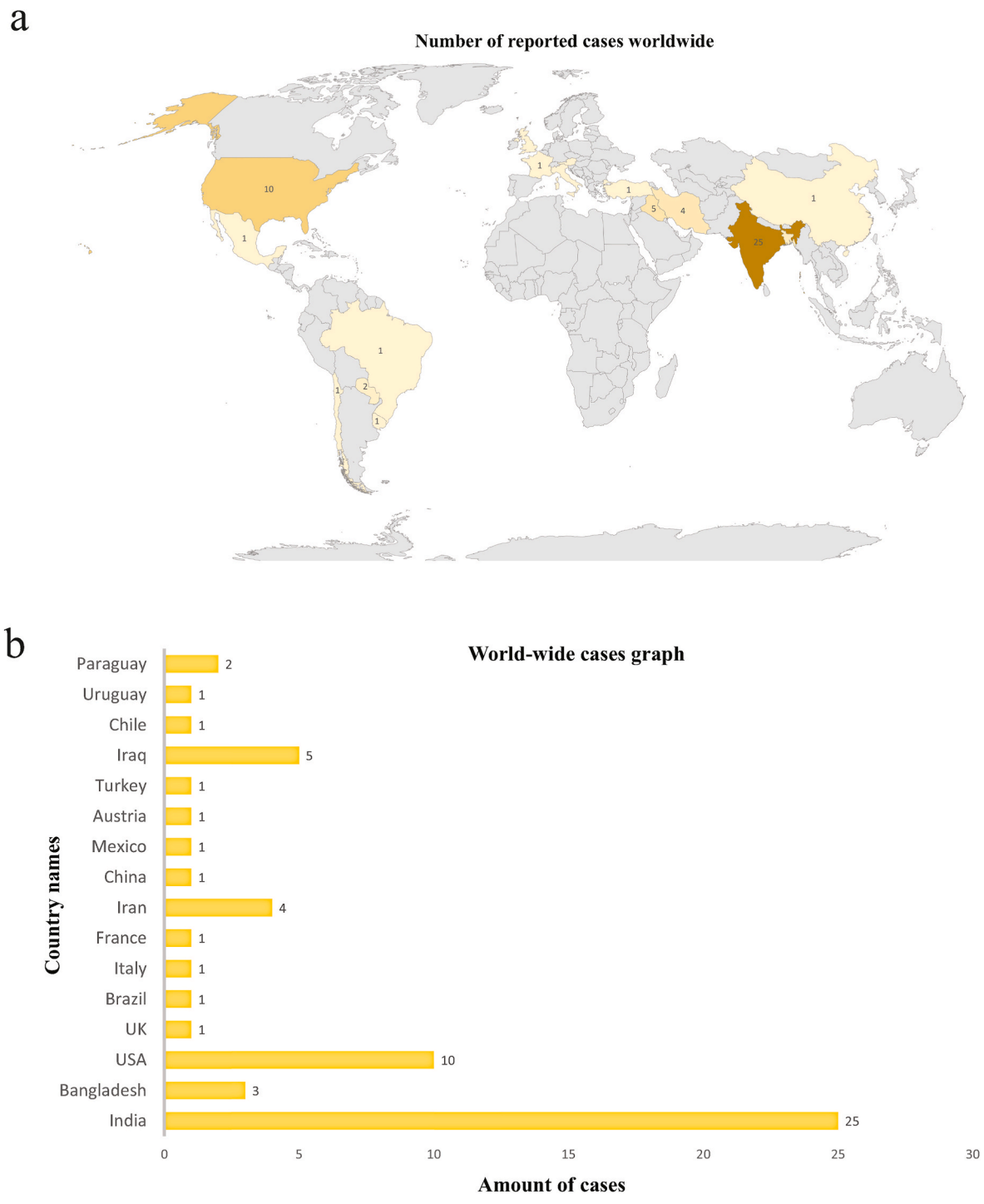


Fig. 1. A global presentation of the number of published case reports of Coronavirus disease 2019 (COVID-19) associated mucormycosis (till May 2021). (a) The color gradient segment of the map indicates the number of absolute cases reported worldwide, where the dark-colored portion represents the higher number of cases, while the light-colored portion represents a smaller number. **(b)** A schematic presentation showing the variation of the number of reported cases in different countries.[20–23]

2. Mucormycosis as COVID-19's deadly companion

The aggravation of COVID-19 in 2020 has effectively crushed the entire world in its first wave, where an enormous number of cases have been noticed including deaths and deterioration. The destruction proceeds in 2021 in the period of second-wave, more in the most exceedingly terrible structure.[18] The flood of COVID-19 in its subsequent wave has additionally left a path of infection and deaths, where the 'black fungus disease' or 'mucormycosis' went with. Mucormycosis is a rare but severe compilation of COVID-19, which may lead to a threat to life.[19,20]

Up to May of 2021, we have dissected around 59 instances of mucormycosis throughout the world, related to the second wave of COVID-19 (Fig. 1). The analytical data of "black fungus disease" specify India with a report of about 42.3% published cases (25/59), followed by the United States of America (10/59), Iraq (5/59), Bangladesh (4/59), Iran (4/59), Paraguay (2/59), and 1 case each from Brazil, Mexico, Italy, UK, China, France, Uruguay, Turkey, and Austria (Table 1). Most patients who torment this obstruction of mucormycosis had some significant comorbidity, by and large, diabetes mellitus, and diabetes ketoacidosis yet contaminations in immunocompetent patients have moreover been conceived.[21,24]

As indicated by several reports till September of 2021, India has been accounted for by 45,435 instances of mucormycosis and is crumbling step by step. The black fungus cases are on the skyscraper in Gujarat alongside Maharashtra with around 7109 and 10,139 cases respectively

Table 1
A brief number of cases of Coronavirus disease 2019 (COVID-19) associated mucormycosis reported worldwide.

Reported Area	Total No. of case	Age/Sex	Underlying Disease		Disease Type	Verified COVID-19	Medicine used for COVID-19	Fungal culture	Clinical Outcomes	Reference
			DM/DKA/ T1DM/ T2DM	Cancer						
India	25	23–78 M-22 F-3	DM-24 (32–78) No- 67 M	No All	Rhino-orbital: 23, 60 Rhino-orbital-cerebral: 40, 38, 51, 45, 56, 78, 67, 56, 37 Rhino-sinusitis: 43, 64, 49, 59 M, 59F Pulmonary: 55, 32, 43, 72 Sino-orbital: 38 Paranasal: 32	Confirmed	Steroid-51, 37, 43, 56, 78, 49, 60, 55, 38, 64, 60, 59F, 72 Tocilizumab-51, 37, 60 Remdisivir-32 M, 51, 37, 43, 56, 49, 55, 62 38, 67, 72, 38, 45 Not applied: 32F, 40, 23	Positive (*Rhizopus* spp.)	Expired-10 Recovered-13 Unchanged-2	5,25,26
Bangladesh	3	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Link 1
USA	10	33–79 M-8 F-2	DM-36, 48, 79, 68 DKA-36, 48, 33, 41 T1DM-60, 41 T2DM-44 No-49, 56	No- 9 (44F Yes)	Rhino-orbital: 33, 60 Rhino-orbital cerebral: 36, 48 Pulmonary: 44, 49, 79, 56 Rhino-cerebral: 41 Cutaneous: 68	Confirmed	Steroid- 36, 44, 48, 49, 60, 41, 79, 56, 68 Tocilizumab-33, 56 Remdesivir-36, 44, 48, 49, 60, 79	Positive all (79 M & 44F *Aspergillus* sp.)	Expired-6 Recovered-3 Unchanged-1	12,14,20,27–32
UK	1	22 M-1	No	No	Pulmonary	Confirmed	Not applied	Positive	Expired	33
Brazil	1	86 M-1	No	No	Gastrointestinal	Confirmed	Not applied	Positive (*Rhizopus* spp.)	Expired	8
Italy	1	66 M-1	No	No	Pulmonary	Confirmed	Not applied	Positive (*Rhizopus* spp.)	Expired	34
France	1	55 M-1	No	Yes	Pulmonary	Confirmed	Not applied	Positive (*Aspergillus* spp.)	Expired	35
Iran	4	40–61 M-2 F-2	DM-44, 54 DKA-No T1DM-No No-40, 61	No-All	Rhino-orbital: 61, 54 Rhino-orbital cerebral: 40 Rhino-sinusitis: 44	Confirmed	Steroid- 40, 44, 54,61 Tocilizumab-No Remdesivir-40, 54	Positive (*Rhizopus* spp.)	Expired-2 Recovered-2 Unchanged-No	24,36,37
China	1	32 F-1	No	No	Rhino-cerebral	Confirmed	Not applied	Positive	Expired	23
Mexico	1	24 F-1	DM-No DKA-24 T1DM-No	No	Rhino-orbital	Confirmed	Not applied	Positive (*Rhizopus* spp.)	Expired	38
Austria	1	53 M-1	No	Yes	Pulmonary	Confirmed	Not applied	Positive (*Rhizopus* spp.)	Expired	39
Turkey	1	56 F-1	DM-56 DKA-56 T1DM-No	No	Rhino-orbital sinusitis	Confirmed	Steroid- 56 Tocilizumab-No Remdesivir-No	Positive (*Rhizopus* spp.)	Expired	40
Uruguay	1	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Recovered	Link 2
Paraguay	2	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Link 3
Iraq	5	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Link 4
Chile	1	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Link 5

M: Male, F: Female, DM: Diabetes mellitus, DKA: Diabetic ketoacidosis, T1DM: Type 1 diabetes mellitus, T2DM: Type 2 diabetes mellitus, COVID-19: Coronavirus disease 2019.

and around 1336 deaths in Maharashtra, and about 708 deaths in Gujarat, brought about by this dark organism (Link 6). Telangana and Madhya Pradesh have been seen with 2638 and 2370 sequentially, where Madhya Pradesh has seen the expiration of 167 individuals. Besides, 1947 cases and 351 deaths in Delhi have been accounted for, close by Haryana with 1764 cases and 268 deaths. Uttar Pradesh, Karnataka, and Rajasthan are confronting the deficiency of liposomal amphotericin B, guaranteeing with around 2477, 3906, and 3621cases distributively, brought about by the verse growth of mucormycosis (Link 7; Link 9). The ongoing report uncovers the passageway of the dark parasite in West Bengal and Punjab with 179 and 158 cases approximately, where 11 deaths of individuals have been reported from West Bengal. Assam and Himachal Pradesh count with the least number of cases and may rise in the upcoming days (Fig. 2). The abrupt acceleration of dark organisms close by COVID-19 leads to the issues for the deficiency of liposomal amphotericin B in numerous states including Goa, Odisha, Kerala, and more. The 'black fungus disease' or 'mucormycosis' have been announced as an 'epidemic' by Rajasthan, Gujarat, and Odisha (Link 6; Link 7; Link 8; Link 9). However, various cases are expanding day by day which may lead towards another disturbance alongside COVID-19.[21,22]

A sudden escalation of mucormycosis is being reported in cases with COVID-19.[22] Many cases reveal the affliction of mucormycosis even while undergoing treatment for COVID-19 (Table 2).

In general, from the referenced cases on mucormycosis related COVID-19 in India, the most effective type of mucormycosis, that is, the sort which holds the more detrimental rate is Rhino-orbital cerebral mucormycosis with about 36%, alongside Rhino-sinusitis mucormycosis with 24%. The Rhino-orbital mucormycosis and Pulmonary mucormycosis holds about 20% and 16% respectively, while the Paranasal type holds the least number of cases with 4% of viability[46–48] (Fig. 3). Trigger off mucormycosis may prompt a deadly rise and could be fatal.

3. *Rhizopus*, the key player for COVID-19 associated mucormycosis

Mucormycosis is driving logically throughout the world, especially in India. The ruling fungal genera Mucorales, especially the *Rhizopus* species is the most well-known growths found in the patients of mucormycosis in both diabetic and non-diabetic COVID-19 patients. *Rhizopus* species appears differently concerning some others from the Mucorales family.[21] Since it is aseptate and making sporangiophores, it is remarkably quick in making and spreading sorts of molds with blackish and a bit of the caramel or brownish sporangia[49] (Fig. 4). Different aspiratory mucormycosis was perceived by going to the parasites with septate hyphae and sporangiophores through direct microscopy or despite fluorescent brighteners from clinical models like sputum, Bronchoalveolar Lavage Fluid (BALF), and so on also, by using the Lactophenol cotton blue (LCB) association in microscopy, the septate hyphal arrangement and the strain of hyphae were analyzed to see the microorganism.[50,51] To confirm the assurance, non-pigmented hyphae showing tissue assault should show up in tissue sections stained with hematoxylin and eosin (HE) staining, Periodic Acid Schiff (PAS), or Grocott-methenamine-silver (GMS).[14,50] The most notable species that cause mucormycosis after COVID-19 in India comprises *Rhizopus oryzae, Rhizopus microsporous, Rhizopus arrhizus, Rhizopus homothallicus*, and some different equally species. These developments now and again impact the immunocompetent, yet rather immunocompromised patients.[21]

In patients with seriously controlled diabetes mellitus, the persistently expanded blood glucose levels will provoke the debilitated neutrophil measure.[52] The parasites increase section through internal breath into the paranasal sinuses and may finally spread to be the sphenoid sinus and immense sinus. However most instances of mucormycosis are sporadic, and a sudden outburst of mucormycosis is ought to be lethal.[53]

3.1. Clinical expressions of the disease

Alongside COVID-19, the major cause for the increasing rate of mucormycosis triggered by *Rhizopus* spp. has been integrated with the upliftment of prevalence of diabetes mellitus (DM) and diabetic ketoacidosis (DKA). Infectious diseases hold up to 12% of all deaths in people with diabetes mellitus.[54–56] DM is a classical fear element for mucormycosis, associated with high ailment and mortality rate in COVID-19, while DKA also stands as an ideal risk factor.[57,58] In recent studies, euglycemic DKA is also being reported in COVID-19 patients.[59] The pervasiveness of type 1 DM and DKA in COVID-19 were much higher compared to the type 2 DM and DKA in the general population.[58] In addition, the utilization of immunosuppressive treatment like glucocorticoids and tocilizumab results in systemic immune adaptions by the infection that paved the way for mucormycosis contamination in patients during COVID-19.[56]

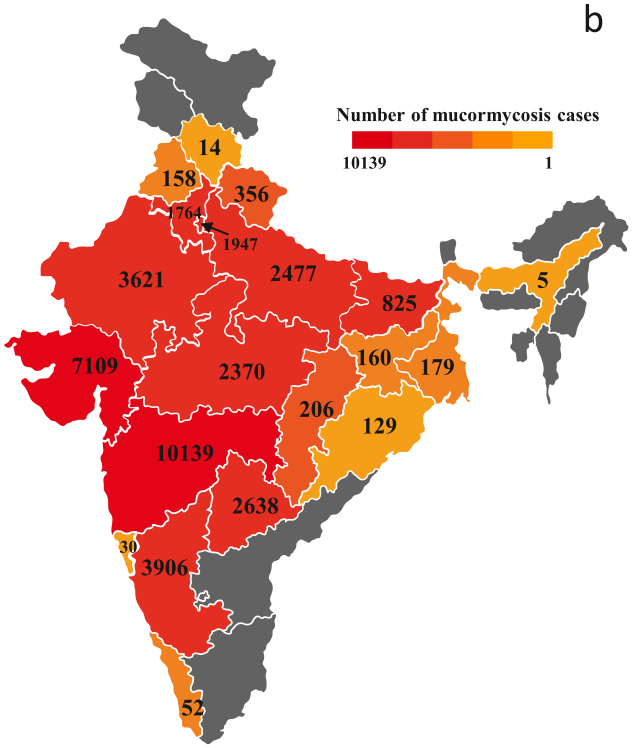
Mucormycosis would also be fatal for the patients who are seriously immunocompromised, likewise in cancer patients or AIDS patients.[21] The infection of mucormycosis targets the region of the nose, sinuses, orbit, central nervous system (CNS), lung (pulmonary), gastrointestinal tract (GIT), skin, jawbones, joints, heart, kidney, mediastinum (invasive type), and abdominal portion.[1,60] It is signalized by the appearance of hyphal invasion of sinus tissue in between a period of fewer than four weeks.[61,62] Fever, headache, coughing, shortness of breath, bloody vomit and, altered mental state are all the primary symptoms of the disease. Moreover, congestion with the nasal release (blackish/bloody), confined pain on the cheekbone, partial facial pain with swelling, blackish discoloration above the bridge of nose or palate, loosening of teeth, diminished or double vision, skin lesion is the severe symptoms for the distinctive types of mucormycosis.[9,21]

In the first instance, the expression of rhino-cerebral mucormycosis is compatible with either sinusitis or periorbital cellulitis and includes eye or facial pain with numbness, followed by the onset of conjunctival suffusion, blurry vision, and soft tissue swelling.[63–67] Fever is inconsistent and might be absent in up to half of cases; white blood cell counts are typically uplifted, as far as the patient has functioning bone marrow.[64,67] Histological features include mycotic invasion of blood vessels, vasculitis with thrombosis, tissue localized necrosis, hemorrhage, and intense neutrophilic infiltrate.[68]

The clinical indications of pulmonary mucormycosis include cough with chest pain and dyspnea.[69] This facilitates the result of inhalation or lymphatic spread. Patients with DKA can also thrive the disease, even though contamination in the patients is less conventional and less volatile than the infectious track that is typically seen in the patients with neutropenia.[69,70] Otherwise, it also arises in the leukemic patients undergoing chemotherapy.[9]

Patients who are at an intense danger of creating cutaneous mucormycosis are those with interruption of the typical defensive cutaneous hindrance. Typically, the factors of mucormycosis are incapable of nauseating intact skin. In immunocompromised and diabetic patients, the cutaneous lesions may also rise due to catheter insertion and insulin injection sites.[71,72] Infected surgical dressings have also been incriminated as a source of cutaneous mucormycosis.[73,74] Mucormycosis in the gastrointestinal tract is rare. It mainly hinders malnourished patients (especially infants or children) and is thought to arise from the ingestion of fungi.[75–80] The most frequently involved sites include the stomach, ileum, and colon. The symptoms are varied and based on the site affected. Fever and hematochezia may also arise, along with lenient abdominal pain and distention related to nausea and vomiting are the best fitted well-known manifestations.[81–83]

a



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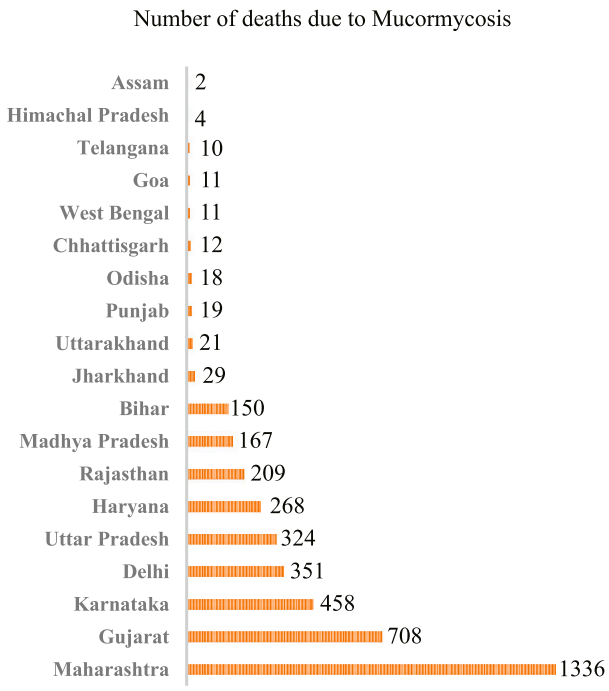

c

States	COVID-19 active cases	Mucormycosis cases
Gujarat	827873	7109
Maharashtra	6640888	10139
Madhya Pradesh	793288	2370
Uttar Pradesh	1710502	2477
Telangana	677546	2638
Rajasthan	954984	3621
Karnataka	2999098	3906
Haryana	771904	1764
Bihar	726259	825
Delhi	1441514	1947
Punjab	603578	158
Uttarakhand	344402	356
Chhattisgarh	1007075	206
Kerala	5178892	52
Jharkhand	349383	160
West Bengal	1620803	179
Odisha	1050760	129
Goa	179307	30
Himachal Pradesh	227753	14
Assam	618042	5

Fig. 2. An illustrative presentation on the number of cases of Coronavirus disease 2019 (COVID-19) associated mucormycosis reported in the different States of India (till September of 2021). (a) The colors provided in the different geographical area represents the variation in the number of cases. **(b)** A schematic presentation on the number of deaths in different States of India due to mucormycosis. **(c)** Up-to-date state-wise statistical indication of COVID-19 cases along with mucormycosis cases of India[21,22] (Link 6; Link 7; Link 8).

Table 2
A precise of cases reported in India on Coronavirus disease 2019 (COVID-19) associated mucormycosis.

Case No.	Age/Sex	Reported Area	Occurrence of fungal colonies during microscopy	Causative Agent	Disease Type	Underlying Disease	Infected internal body parts	Symptoms	Clinical outcomes	Reference
Case-1	32/F	Mangalore	Positive	*Rhizopus* spp.	Paranasal Mucormycosis	Diabetes mellitus, left eye complete ptosis, facial problem	Sinus and orbit	Orbital apex syndrome Rapidly lost eye vision	Recovered but no improvement in vision	[41]
Case-2	60/M	Mumbai	Positive	*Rhizopus* spp.	Rhino-orbital Mucormycosis	Diabetes mellitus, Lung disease	Sinus and orbit	orbital swelling, headache, nosebleed	Expired	[42]
Case-3	38/M	Mumbai	Positive	*Rhizopus oryzae*	Sino-orbital Mucormycosis	Diabetes mellitus	Sinus and orbit	Swelling and pain in the left eye	Recovered	[5]
Case-4	72/M	Hyderabad	Positive	*Rhizopus. oryzae*	Pulmonary Mucormycosis	Diabetes mellitus, hypertension	Lungs	Streaky hemoptysis	The patient is not improving	[43]
Case-5	40/F	Mangalore	Positive	*Rhizopus* spp.	Rhino orbital cerebral Mucormycosis	Diabetes mellitus	Sinus, orbit, and CNS	Swelling of the left eye and facial pain, rhinitis	Recovered	[15]
Case-6	38/M	Bangalore	Positive	*Rhizopus oryzae*	Rhino orbital cerebral Mucormycosis	Diabetes mellitus	Orbit, sinus	Right eye pain and chemosis	Expired	[26]
Case-7	51/F	Mumbai	Positive	*Rhizopus oryzae*	Rhino orbital cerebral Mucormycosis	Diabetes, Hypothyroidism	Eye, sinus, and CNS	Left side facial pain, nose block, periorbital pain, and headache	Recovered	[26]
Case-8	45/M	Puducherry	Positive	*Rhizopus oryzae*	Rhino orbital cerebral Mucormycosis	Diabetes mellitus, Hypertension, CKD	Eye damage, sinus, and CNS	Impairment of right eye vision	Recovered	[26]
Case-9	56/M	Bangalore	Positive	*Rhizopus oryzae*	Rhino orbital cerebral Mucormycosis	CKD, diabetes, hypertension, hyperthyroidism	Eye conjunctiva, brain	Right eye swelling	Expired	[26]
Case-10	78/M	Bangalore	Positive	*Rhizopus oryzae*	Rhino orbital cerebral Mucormycosis	Diabetes and hypertension	Sinus, orbit, and CNS	Holocranial headache	Expired	[26]
Case-11	43/M	Bangalore	Positive	*Rhizopus oryzae*	Rhino-sinusitis Mucormycosi	Diabetes mellitus, CLD	Sinus, nasal passages, oral cavity, and brain	Dryness and cresting in the nasal cavity	Recovered	[26]
Case-12	60/M	Delhi	Positive	*Rhizopus arrhizus*	Rhino-sinusitis Mucormycosis	Diabetes mellitus, deranged kidney function	Sinus and brain	Periorbital swelling, chemosis, restricted eye movement	Expired	[44]
Case-13	64/M	Delhi	Positive	*Rhizopus microsporus*	Rhino-sinusitis Mucormycosis	Diabetes mellitus, renal function failure	Sinus, nasal passages, oral cavity, and brain	Proptosis of the eye with Periorbital discoloration, blackening of the middle turbinate.	Expired	[44]
Case-14	67/M	Not Reported	Positive	*Rhizopus oryzae*	Rhino orbital cerebral Mucormycosis	Hypertension	Cornia, conjunctiva, eyelids, optic nerve damage	High fever, dizziness, blurred vision	Recovered	[45]
Case-15	49/M	Not Reported	Positive	*Rhizopus homothallicus*	Rhino-sinusitis Mucormycosis	Diabetes mellitus, problem in breathing	Sinus, brain, and nasal passages	High fever, facial swelling	Recovered	[45]
Case-16	23/M	Not Reported	Positive	*Rhizopus oryzae*	Rhino-orbital Mucormycosis	Diabetes mellitus, hypertension	Sinus and orbit	High fever, headache, periorbital pain, facial pain	Expired	[45]
Case-17	59/F	Delhi	Positive	*Rhizopus arrhizus*	Rhino-sinusitis Mucormycosis	Diabetes	Sinus and brain	High fever, facial swelling, blackening of turbinate	Recovered	[45]
Case-18	62/M		Positive						Expired	[45]

(*continued on next page*)

Table 2 (*continued*)

Case No.	Age/Sex	Reported Area	Occurrence of fungal colonies during microscopy	Causative Agent	Disease Type	Underlying Disease	Infected internal body parts	Symptoms	Clinical outcomes	Reference
		Not Reported		*Rhizopus oryzae*	Rhino orbital Mucormycosis	Diabetes mellitus, High pressure	Sinus and orbit	Periorbital pain, blurred vision, and headache		
Case-19	43/M	Not Reported	Positive	*Rhizopus oryzae*	Pulmonary mucormycosis	Diabetes mellitus, problems in renal area	Lung	Facial swelling, infection in the lung, high fever	Recovered	[45]
Case 20	32/M	Hyderabad	Positive	*Rhizopus arrhizus*	Pulmonary Mucormycosis	Diabetes mellitus	Lung	High fever, nasal tract infection, headache, infection in lung	Recovered	[45]
Case-21	60/M	Mumbai	Positive	*Rhizopus oryzae*	Rhino orbital Mucormycosis	Diabetes mellitus	Sinus and orbit	Periorbital pain, blurred vision	Expired	[22]
Case-22	55/M	Chandigarh	Positive	*Rhizopus* spp.	Pulmonary Mucormycosis	Diabetes mellitus, End-stage kidney disease	Lung	Facial swelling, infection in the lung, high fever	Recovered	[22]
Case-23	59/M	Delhi	Positive	*Rhizopus* spp.	Rhino sinusitis Mucormycosis	Diabetes mellitus, High pressure, Coronary artery disease	Sinus and brain	High fever and facial swelling, blackening of turbinate	Expired	[25]
Case-24	56/M	Bangalore	Positive	*Rhizopus oryzae*	Rhino orbital cerebral Mucormycosis	Diabetes mellitus	Cornia, conjunctiva, eyelids, optic nerve damage	Right eye pain and gradual loss of vision	Loss of follow up	[26]
Case-25	37/M	Not Reported	Positive	*Rhizopus oryzae*	Rhino orbital cerebral Mucormycosis	Diabetes mellitus	Cornia, conjunctiva, eyelids, optic nerve damage	Pain and bleeding from gums	Recovered	[26]

M: Male, F: Female, CKD: Chronic kidney disease, CLD: Chronic liver disease, CNS: Central nervous system.

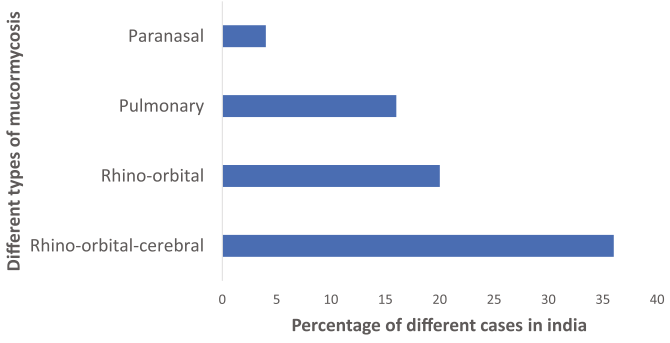

Fig. 3. An evanescent theory on the types of mucormycosis presenting their efficacious side on the Coronavirus disease 2019 (COVID-19) associated mucormycosis patients. The diagram represents rhino-orbital cerebral (36%) mucormycosis is the highest reported type in India followed by the rhino orbital (20%), pulmonary (16%), and paranasal (4%).[21,22]

4. Molecular mechanism: the panoramic story of COVID-19 associated mucormycosis

4.1. Exposition: Preface of the story

The attendance of Diabetes mellitus (DM), whether with or without Diabetic ketoacidosis (DKA), enhances the chance of acquiring mucormycosis, and DM is frequently linked to enhanced COVID-19 intensity. Meanwhile, corticosteroid use is regularly linked with uncontrolled hyperglycemia and the commencement of DKA. Acidosis causes a low pH, which is ideal for mucor spores to grow. Furthermore, use of steroid decreases the phagocytic nature of WBC (both first and second-line defensive mechanisms), impairs bronchoalveolar macrophage ingestion, migration, and phagolysosome fusion, and makes a diabetic patient more prone to mucormycosis.[22]

4.2. Crisis period of the story

According to a well-known and established hypothesis about the pathogenesis of DM, elevated levels of glucose in the muscle, and adipose tissue induce cellular hypoxia, endoplasmic reticulum (ER) stress, enhanced discharge of reactive oxygen species (ROS), free fatty acids (FFA), and cytokine production. Interleukin-1 (IL-1β) and tumor necrosis factor (TNF) are released by hypertrophic cells in adipose tissue, along with different chemokines. TNF-α recruits M1 macrophages, and

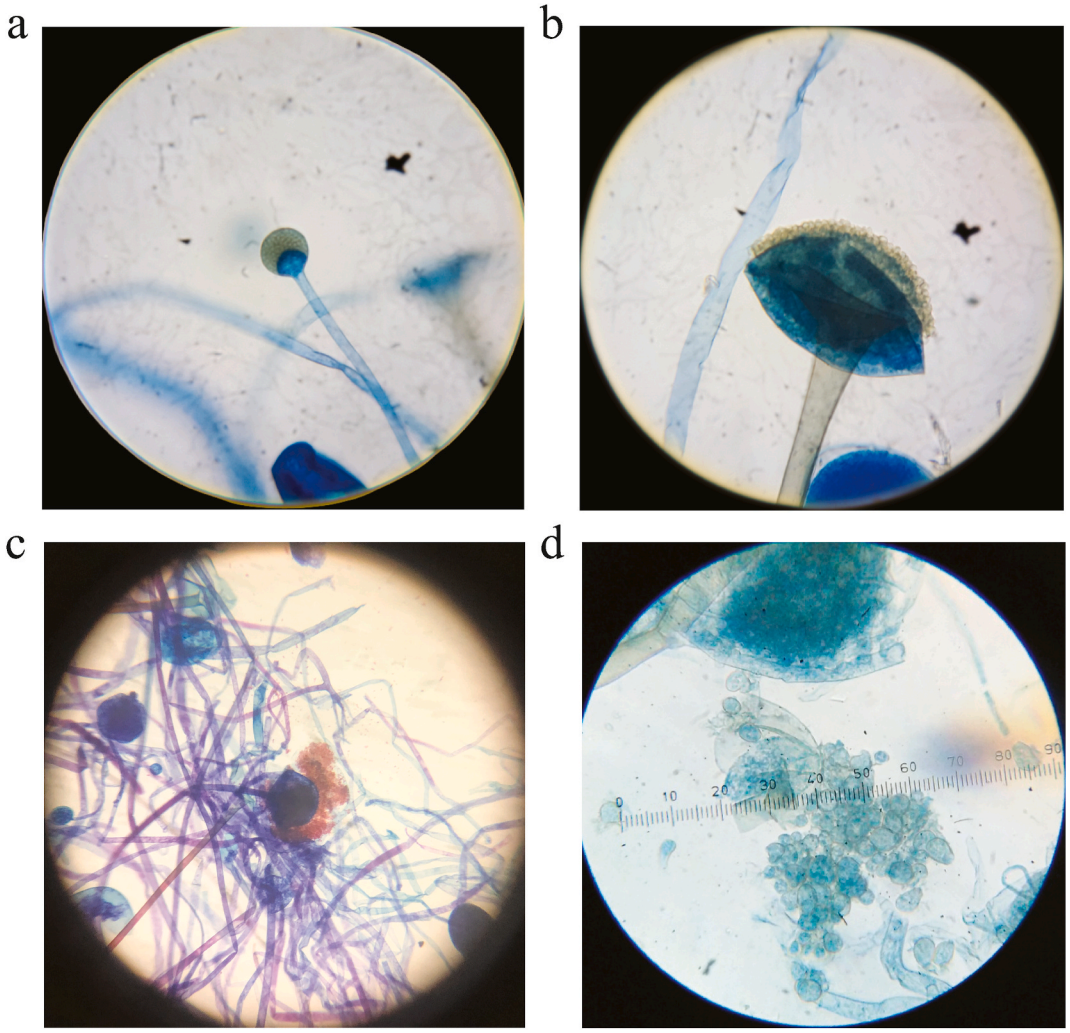

Fig. 4. Microscopic view of *Rhizopus* spp. under Lactophenol cotton blue (LCB) mount. (a–b) Compound microscopic view of *Rhizopus* sp. showing columella and brownish sporangia under ca.×100 and ca.× 450 magnification, respectively. **(c)** Compound microscopic view of *Rhizopus* sp. showing the hyphal region under ca.×100 magnification. **(d)** Compound microscopic view of *Rhizopus* sp. showing sporangiospores under ca.×450 magnification.

its activation produces more pro-inflammatory cytokines (most notably IL-1β) that cause chronic inflammation and the employment of additional M1 macrophages. FFA is also detected by TLR in the tissue cells, initiating JNK-AP-I and IKK-NFKB signalling.[84–86] The utterance and discharge of pro-inflammatory cytokines are enhanced consequently, which promotes the native inflammatory state. In diabetes individuals, M1 macrophages infiltrate the tissue, producing a pro-inflammatory M1 macrophage response rather than a regulating M2 macrophage response. Because M2 macrophages seem to be better able to trigger and then destroy fungal cells, penetration of diabetic tissue with M1 macrophages could provide to *Rhizopus* spp. impedance to phagocytosis.[87]

Several cellular level injuries like endothelial damage, endotheliali-tis, lymphopenia, thrombosis, and a drop-down in the degree of $CD4^{+}$, $CD8^{+}$, and T-cells levels are frequently caused by COVID-19 which is ultimately putting the patient at risk of secondary or opportunistic fungal infection.[22]

Proteins like ferritin and transferrin show excessive glycosylation due to the effect of hyperglycemia which ultimately reduces their iron affinity.[88] Furthermore, the low pH environment in the blood vessels severely limits transferrin's ability to chelate iron in the presence of an acidotic state triggered by the generation of ketone bodies (e.g., β-hydroxybutyrate [BHB]).[89] Thus, the availability of the free iron in the blood vessel is triggered and a combined effect of free iron, glucose, and BHB activates the hyphal expansion of the fungus.[90,91]

The fasting condition induced by a lack of insulin causes the catabolism of amino acids and triacylglycerols (TAGs), deposited in adipose tissue to become active as an energy source in diabetic patients. In serum, due to limited lipolysis, the concentrations of free fatty acids and glycerol, are much higher whereas the concentration of alanine is much higher due to muscle catabolism. Excess glucagon and insulin insufficiency stimulates gluconeogenesis, which uses those alanines and glycerol as substrates. Glucagon also advances the transformation of free fatty acids to ketones in the mitochondria. Insulin inhibits the transfer of the derivatives of free fatty acid to the matrix of mitochondria in normal conditions, but ketogenesis continues in the deficiency of insulin.[85] Numerous ketone bodies are produced by virtue of TAG metabolism, influencing serum pH and causing the malfunction of numerous serum enzymes. Few instances, such as hemoglobin and transferrin, remain protonated and unable to transport Fe^{+3} at a pH of 6.88–7.3, resulting in a higher amount of Fe^{+3} accessible in serum in diabetic patients. *Rhizopus* has a ketone reductase enzyme that enables the fungus to develop in this acidic condition apart from using the free Fe^{+3} in these patients.[92,93] The acidosis produced by *Rhizopus* spp. affects other host enzymes, which hold a direct impression on chemotaxis and phagocytosis. Reduced iron levels have also been shown to promote the M1 pro-inflammatory LPS-induced response, suggesting that additional mechanism contributes to the dissemination of an adverse feedback to fungal allowance.[94]

4.3. Rising period of the story

Free iron is another excellent resource for mucormycosis. According to several studies, iron plays a major function in *Rhizopus* and it is taken from the host via two methods, either siderophores (iron chelators) or high-affinity iron permeases.[95–98] Fungi battle with the host for the free iron in the siderophore system. Intrinsic and extrinsic siderophores are the two major types of fungi siderophores. Speaking of *Rhizopus*, both forms of siderophores are utilized. The major intrinsic siderophore, found in *Rhizopus*, is Rhizoferrin. It absorbs iron from outside the cell environment via a receptor-conciliated and energy-reliant method. Thirteen potential siderophore permeases are found after the genome-sequencing investigation of *R. oryzae* which could act as receptors for different siderophores. According to numerous protein crystallography experiments, rhizoferrin has a diaminobutane backbone connected to two citric acid residues with an R, R arrangement encircling a chiral centre.[57,99]

Another consideration for a better phagocytic response is reactive oxygen species (ROS). Owing to insulin resistance, hyperglycemia persists in people with diabetes, and in an attempt to lower glucose levels, glucose metabolism and secondary lipolysis are elevated via oxidative phosphorylation. Low pH in patients with diabetic ketoacidosis (DKA) makes more vulnerability to mucormycosis as a result of the summed-up oxidative climate which influences glutathione to remodel through the GSH/GSSG compound cycle. Advanced glycation end products (AGEs) and ROS produced by enhanced glucose metabolism cumulate in organs and tissues, causing typical micro and macrovascular changes in diabetic patients directing to an enlarged vulnerability to a *Rhizopus* infection.[100,101] Due to inadequacy of the cofactor NADPH, down-regulation of the major antioxidant system of glutathione (GSH/GSSG), which is the prime requirement for the reconstruction of reduced glutathione, ultimately reduces the ability of the patient to control the oxidative stress. The polyol route for glucose metabolism consumes NADPH quickly, resulting in a deficit of NADPH. Oxidative stress triggers inflammation through the NF-kB and TLR receptors, resulting in a long-term chronic inflammatory state.[57,101]

4.4. The climax of the story: The interaction between GRP78 and CotH3

In transformed fibroblasts, the production rate of a particular protein was increased when the reduction of glucose was caused. Later on, that particular protein was discovered as glucose-regulated proteins (GRPs). GRP78 or glucose-regulated protein has a molecular weight of 78-kDa, was first identified as a heat shock protein that has a role in stress-related responses.[102] It is also known as immunoglobulin-binding protein (BiP) or HSP5a and is mostly found in the lumen of the endoplasmic reticulum (ER) and produced in mammalian cells. The HSP5a gene, which is found on chromosome 9q34, encodes GRP78. GRP78 is mostly found in the ER, although it has also been found in the cytoplasm, mitochondria, nucleus, plasma membrane, and secreted, even though it is primarily responsible for engaging endogenous cytoprotective mechanisms.[103] The nucleotide-binding domain (NBD) or ATPase and substrate-binding domain (SBD) or protein/peptide-binding domains are the two main functional domains of GRP78.[104–106] The function of this protein is controlled by the allosteric ATPase cycle in which the binding with ATP and hydrolyzation of ATP is performed by NBD whereas the SBD performs the job of bindings with polypeptides.[104,105,107] GRP78 has long been believed to be a molecular chaperone having a place with the HSP70 family that directs the unfolded protein reaction (UPR) to control ER stress and assumes a critical part in protein collapsing and quality control, just as misformed protein degradation.[108,109]

GRP78 expression has recently gained importance due to its translocation to the cell membrane's surface (csGRP78) during ER stress,[110] where it serves as a receptor and regulator in cell indicating by forming complexes with extracellular ligands and proteins attached to the cell surface.[111–113] Recent Research reveals that hyperglycemia behaves like a stress trigger in ER which simultaneously initiates the overexpression of the GRP78 protein, based on the glucose concentration. MTJ-1 chaperone-mediated mechanism helps to translocate these GRP78 proteins from the ER to the cell surface.[114] Likewise, overexpression of csGRP78 has been found to play a crucial role as an entrance receptor for various pathogens, including the Ebola virus, Dengue virus, Coxsackievirus, and the new SARS-CoV2 virus, and other viruses and *Rhizopus* spp. as well.[115–117]

Rhizopus spp. interact with various receptors of epithelial cells of alveolar and nasal origin. When Rhizopus spp. infect nasal epithelial cells, csGRP78 is overexpressed, but not in alveolar epithelial cells. In addition, it was discovered that *Rhizopus* spp. interrelate with alveolar epithelial cells by binding to integrin-1 rather than csGRP78. Subcellular factors, like iron, glucose, and DKA trigger the excessive production of csGRP78 only in nasal epithelial cells and subsequently enhance the pathogenicity of *Rhizopus* spp.[57]

Following the discovery of GRP78 as a required receptor for the invasion of the species of Mucorales,[115] the hunt for a possible ligand led to the discovery of CotH in Mucorales.[116] As a result, in a wide spectrum of Mucorales species, the utterance of the CotH1, CotH2, and CotH3 genes has been identified. Nonetheless, research data has suggested that CotH3 is mostly produced in *R. oryzae* germinations and has a better ability to attach and so penetrate endothelium and nasal epithelial cells in the DKA environment.[95,116,118,119] On the other hand, CotH7 is the primary ligand that interrelates with integrin-1 of alveolar epithelial cells in the pulmonary mucormycosis, and it is not closely linked to CotH3 (50% amino acid identity).[118]

In Mucorales, csGRP78 binds particularly with spore coating homolog proteins (CotH), facilitating invasion and injury to endothelial cells.[115,116,118,119] By nature, CotH protein is a type of protein kinase and a member of a vast family of spore coating proteins. It has diversified functions. It is essential for protein assembly in the inner layer of the spore-coat. During sporulation, this protein is produced and shows its activity. ATP-dependent autophosphorylation and successive phosphorylation of serine residues of CotG and CotB proteins regulate its activity. The half-life of CotH is only four to 5 h. Its concentration drops quickly when the structural gene's transcription is turned off. Recent findings show its essential role in spore germination of many human pathogens like spore-producing fungi such as *Rhizopus oryzae* and the expression of many bacterial strains like *Bacillus anthracis*.[116,120,121]

The appearance and interaction of GRP78 and CotH leads to increased fungal interference and consequent endothelial injury in vitro.[91,115] As the iron chelation fused with pH reversal by sodium bicarbonate protects endothelial cells from *Rhizopus*-mediated invasion and injury,[91] it emerges that BHB-related acidosis has a straight effect on both GRP78 and CotH expression and an indirect effect by compromising transferrin's ability to chelate iron. Importantly, host cells with higher BHB, produced as a result of DKA, have lower blood pH, higher accessible serum iron, higher GRP78 expression in focussed organs (e.g., lungs and sinuses), and are more susceptible to mucormycosis.[91,115]

Thus, the extraordinary affectability of DKA patients to mucormycosis is clarified by the special communications of GRP78 and CotH proteins, just as their expanded articulation under hyperglycemia and ketoacidosis. Treatment with anti-GRP78 or anti-CotH antibodies protects DKA and neutropenic mice against mucormycosis, emphasizing the relevance of GRP78/CotH protein interactions in the progress of mucormycosis.[115,116,122] The discovery that reversing ketoacidosis in *Rhizopus*-infected animals by administering sodium bicarbonate (instead of insulin) enhances survival is also potentially clinically relevant.[91] Reversal of accelerated fungal expansion, reconstruction of immune function, and terminating of fungal invasion of host tissues are thought to be the causes of this protection. The activity of GRP78/CotH interactions in the neutropenic host, the other main patient category prone to mucormycosis, is currently unknown.[122,123]

4.5. Falling action of the story

The processes that increase the interaction of invading fungus with endothelial/epithelial cells are beginning to gain a toehold, and they represent a key stage in the pathogenesis of diabetes-associated mucormycosis.[115,116,118] Thus, the DKA environment, high glucose, iron, and Bhydroxy butyrate (BHB) as the vital ketone body promote fungal development by promoting CotH3 expression.[124] The surface translocation of the GRP78 protein, which copes with endoplasmic reticulum stress occurred by hyperglycemia and an acid milieu, assists a tissue stage favorable to *Rhizopus* spp. establishment. Iron is released from sequestrated protein transferrin by glycosylation mechanisms in the same tissular niche. As a result, high glucose concentrations, free iron availability,[125] and acerbic microenvironment boost CotH expression on the fungal cell surface facilitating GRP78/CotH3 contact for endothelial/epithelial invasion and fungal spread.[124] The fungus must interrelate with its basement membrane after infecting the apparent nasal epithelium because the spores and stem cells from germ tubes adhere to extracellular matrix constituents. The scrutiny of *Rhizopus* spp. sticking to plates coated with collagen IV and laminin supports this theory.[126]

4.6. Resolution: The final consequences

Meanwhile, endothelial cells keep on creating GRP78 in all cubicles, and the hypha can connect with these proteins on the basal side where the existence of reticulin filaments is surpassed, permitting it to secure and outdo this region to later collaborate with GRP78 communicated on endothelial cells' luminal surface. When fungi become actualized in the lumen of blood vessels, they activate the extrinsic coagulation pathway, which causes cell injury and, as a result, the thrombus formed. This causes ischemia and prolonged hypoxia, resulting in tissue infarction and necrosis (Fig. 5). Finally, the microenvironment has changed and the disease has been established on the body.[57]

5. Proposed modes of investigation for COVID-19 associated mucormycosis

To date, there are no pathognomonic hematologic changes. Elevated white blood cell counts and acute-phase reactant levels indicate the abnormalities that are found reflect underlying predisposing conditions (e.g., diabetic ketoacidosis) and general indications of fungal infection. Blood cultures are virtually always negative. Plain orbit or sinus radiography is not a reliable investigation for this disease.[95,127]

Computed Tomography (CT) analysis indicates the extent of orbital and cranial involvement and progression of the disease. Magnetic resonance imaging (MRI) is also helpful by showing T2-weighted MR images, which demonstrate intracerebral extension while on the other hand, contrast-enhanced MRI scans give us a demonstration of the perineural spread of disease.[128]

Angiography or surgical exploration is necessary for areas of anatomic complexity. Biopsy with histopathologic examination remains the most sensitive and specific modality for definitive diagnosis. Microscopic investigation shows that aseptate hyphal elements of the species belong to the order Mucorales are wide (ranging from 6 to 30 μm), thick-walled, ribbon-like, and showing branch at right angles,[42] whereas the hyphae of *Aspergillus* and, *Fusarium* are comparatively thinner, highly septate and showing branch at acute angles (Fig. 6). The width of the fungus and its ribbon-like shape are the most distinctive characteristics for identifying mucormycosis.[129,130] Schiff or hematoxylin and eosin staining can be used to better visualize the Mucorales; they do not stain as well as methenamine silver.[130]

Histopathology is used to identify the Mucorales, but species identification is limited to culturing. Imaging techniques are used to investigate the condition's advancement and severity. For example, fungal sinusitis that is different from bacterial sinusitis is the most usual finding on CT or MRI scans of the head and sinuses of a patient with rhino-orbital mucormycosis. MRI is more sensitive (by approximately 80%) than CT in the detection of orbital and CNS disease.[131] Nasal endoscopy is an excellent diagnostic method for determining the presence of mucormycosis, while the MRI findings are very useful and significant to show the spread of mucormycosis in different regions as a supportive example to make it clinically significant but these findings will be varying according to the case-by-case basis (Fig. 7).

The polymerase chain reaction (PCR) is used as a current diagnostic tool in the research of mucormycosis,[132] however, it has not yet been licensed by the U.S. Food and Drug Administration (FDA) for this purpose and it is a rare find.[9]

6. Current therapeutics for COVID-19 associated mucormycosis

As significant trouble, the prevalent COVID-19 spreads worldwide.[25,133] While various treatment options are estimated, at that time systemic glucocorticoids are shown to enhance the survival rate of COVID-19.[25,134] Glucocorticoids are not too expensive, available widely, and are shown to decrease fatality in COVID-19 patients with hypoxemia.[9,25] Unfortunately, the extensive use of glucocorticoids can develop secondary fungal infections like mucormycosis.[25] The medical diagnosis of mucormycosis requires treatment quickly, as the fungal invasion advances rapidly.[50,135–137] Simultaneously, the therapeutics of mucormycosis should be established as soon as possible, to reverse underlying risk factors.[138]

Surgical debridement (FESS or Functional Endoscopic Sinus Surgery is a minimally invasive technique used to restore sinus ventilation and normal function and/or orbital exenteration) not only decreases the burden of the disease but also permits better percolation of intravenous medical drugs. It reduces further disease spreading and permits to allow intraoperative diagnosis of necrotic tissue with applicable characteristics to provide the sample for microbiological and histopathological confirmation.[139,140] But prompt initiation of medication therapy and instant reversal of underlying risk factors are always the better alternatives to surgical debridement because it is crucial to maintain a high index of suspicion in patients who are at risk for mucormycosis at all times.[138]

Antifungals can also play an important role along with surgical debridement. The guidelines, accepted globally in 2019 for the management and diagnosis of mucormycosis by Mycoses Study Group Education and Research Consortium (MSGERC) and the European Confederation of Medical Mycology (ECMM) strongly prescribe surgical treatment if possible with the addition of systemic treatment of antifungals..[141] Liposomal Amphotericin B, Posaconazole and Amphotericin B lipid complex oral suspension can be treated as first-line antifungal agent monotherapy and Isavuconazole can be assisted like salvage therapy.[141,142] Irrigation of sinuses and orbit with(1 mg/ml) Amphotericin B improves the local drug concentration and is shown to enhance outcomes.[143] Intra-orbital and Retrobulbar injection in respect to Amphotericin B are also given in those patients who have no ability for surgical debridement (the dose of anesthesia along with retrobulbar injection is 1 ml of three 3.5 mg/ml).[139,143,144] The recent guideline of MSGERC and ECMM for mucormycosis management recommends liposomal amphotericin B (L-AMB), the dose is 5–10 mg/kg every day.[141,145] Adults and children are often administered to treat mucormycosis at start-up doses of 1 mg/kg daily for Amphotericin B deoxycholate (d-AMB) and 5 mg/kg daily for L-AMB and Amphotericin B lipid complex (ABLC).[127] The dose of 5 mg/kg is used to recommend when the implication of the nervous system is absent.[25,141] Amphotericin B has potential renal toxicity so that the dosage should be adjusted between 0.5 mg/kg/day and 1.5 mg/kg/day by the condition of the patient as well as disease. Hyperbaric oxygen (HBO) therapy should also be used in case of aggressive infection.[9]

For the hyperglycemic patient, the early treatment of liposomal amphotericin B and if necessary surgical treatment is needed.

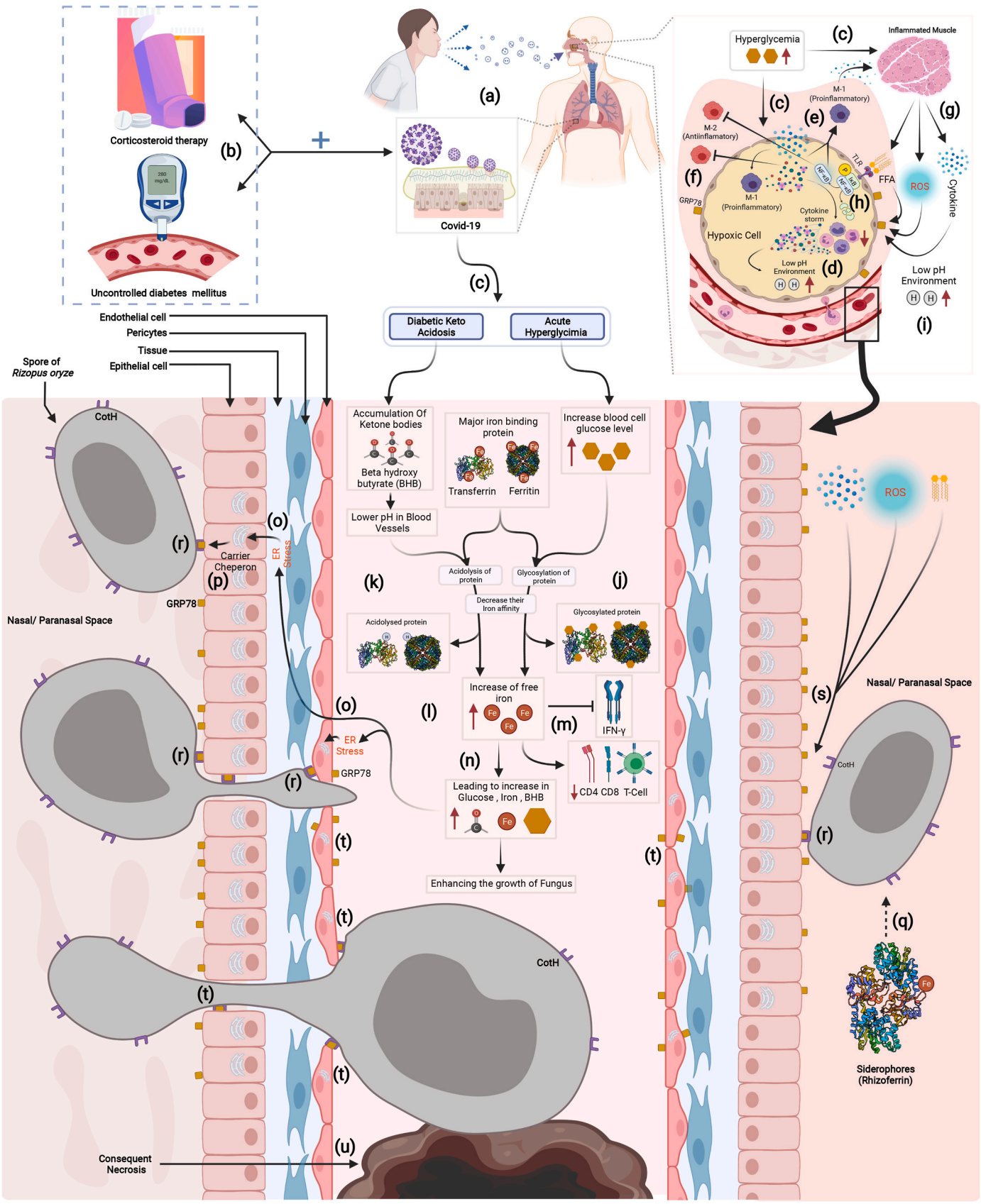

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Fig. 5. Diagram pictured the planned mechanisms for the immunopathogenesis of COVID-19 assisted mucormycosis in the immunocompromised diabetic individual[22,57,124] **(Created with BioRender.com). (a)** In COVID-19 severity, **(b)** uncontrolled diabetes mellitus and overdrive of Corticosteroid drugs increases the vulnerability to Mucorales infection due to diabetic ketoacidosis (DKA) and hyperglycemia. **(c)** An elevation in the glucose level of the adipose tissue induces endoplasmic reticulum (ER) stress, cellular hypoxia, enhanced discharge of free fatty acids (FFA), reactive oxygen species (ROS), and generate cytokine storm. **(d)** A diversified range of the cytokines like interleukin-1 (IL-1β), tumor necrosis factor (TNF), and various types of chemokines are released to the cellular hypoxic environment. **(e)** These cytokines especially TNF-α recruits the proinflammatory M1 macrophages and **(f)** inhibits the activity of anti-inflammatory M2 macrophages. **(g)** The activated M1 macrophage again discharges more pro-inflammatory cytokines like IL-1β, FFA and generates ROS. **(h)** These FFA are also detected by TLR-4 in the tissue cells, initiating JNK-AP-I and IKK-NFkB (nuclear factor-kappa B) signalling. **(i)** Simultaneously, diabetic ketoacidosis (DKA) causes a low pH environment which ultimately enhances the cellular H^+ ion level. **(j)** Due to the activity of hyperglycemia, iron-scavenging proteins like ferritin and transferrin show increased glycosylation in the blood vessel, which lowers their iron affinity. **(k)** Furthermore, in the attendance of an acidotic condition promoted by the creation of ketone bodies (e.g., β-hydroxybutyrate [BHB]), the low pH environment in the blood vessels substantially restricts transferrin's ability to chelate iron. **(l)** As a result, the accessibility of free iron in the blood vessel is stimulated whereas **(m)** the counts of IFN-γ, $CD4^+$, $CD8^+$, and T-cell are sharply declined. **(n)** A combination of free iron, glucose, and BHB triggers epithelial fungal adhesion and tissular hyphal growth or opportunistic fungal infection. **(o)** This combination causes a stress response in ER, which drives to overexpression of the GRP78 protein. **(p)** The MTJ-1 chaperone aids in the translocation of GRP78 proteins from ER to the cell surface. **(q)** Fungi battle with the host for the presence of iron in the siderophore system. **(r)** High glucose concentrations, free iron availability, and an acid microenvironment boost CotH expression on the fungal cell surface, facilitating GRP78/CotH3 contact for epithelial/endothelial invasion and fungal spread. **(s)** The connection between GRP78 and CotH is additionally aided by ROS, FFA, and cytokines. **(t)** Meanwhile, endothelial cells pursue to generate GRP78 in all partitions, and the hypha can connect with these proteins on the basal side and become internalized in the lumen of blood vessels. **(u)** They produce cell damage, thrombus formation, ischemia, prolonged hypoxia, tissue infarction, and finally necrosis by activating the external coagulation pathway.

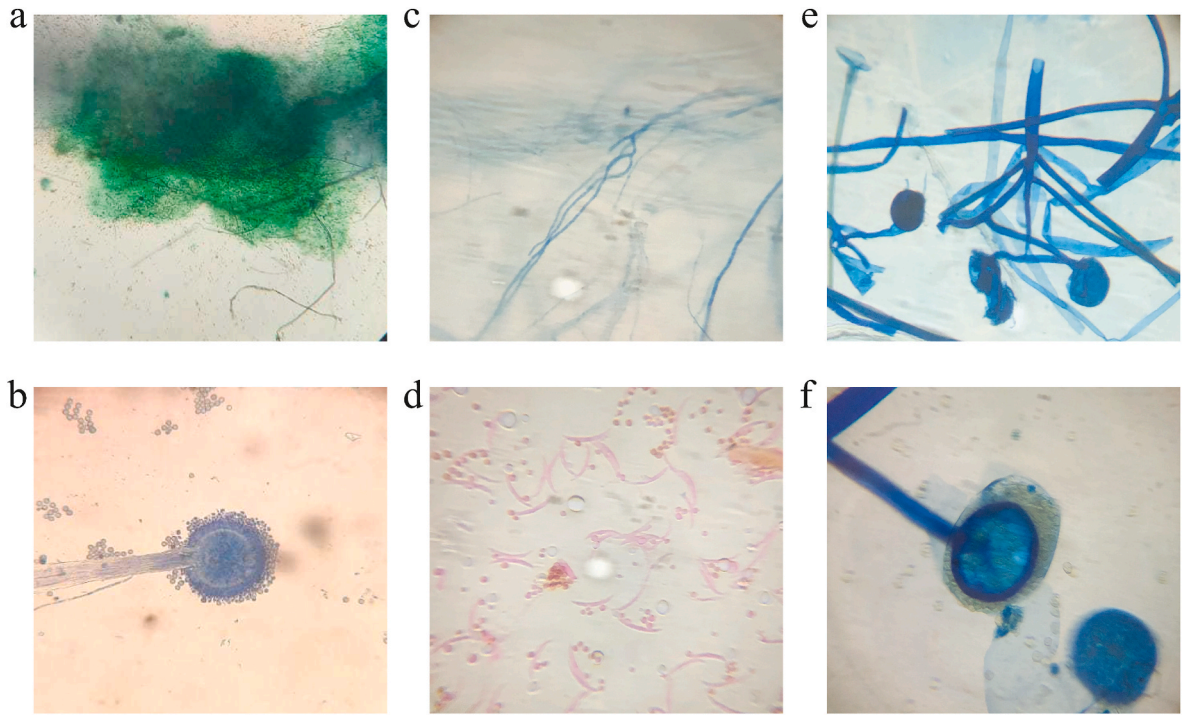

Fig. 6. Compound microscopic view of different types of fungal species. (a–b) Compound microscopic view of *Aspergillus* sp. showing perpendicular hyphal branching pattern under ca.×100 and ca.× 450 magnification, respectively. **(c-d)** Compound microscopic view of *Fusarium* sp. showing conidia with conidiospores and dichotomous hyphal branching pattern under ca.×100 and ca.× 450 magnification, respectively. **(e-f)** Compound microscopic view of *Rhizopus* sp. showing perpendicular hyphal branching pattern under ca.×100 and ca.× 450 magnification, respectively.

Hyperglycemia is annoyed with COVID-19 effective therapy, namely glucocorticoids. Multi-organ dysfunction and co-existing Acute Respiratory Distress Syndrome (ARDS) prevent timely testing and diagnostic imaging.[25,34] The hospitals are overburdened by patients of COVID-19, and diagnostics after those surgeries can be curtailed significantly.[34] Hence, the mortality of COVID-19 associated mucormycosis may higher than non-COVID patients with mucormycosis because of the immunosuppressed condition of the patient, presence of acute hyperglycemia, and necessarily use of glucocorticoids to treat the severity of COVID-19.[49,146,147] Thus, in moderate COVID-19 cases (absence of hyperglycemia), the huge dose of glucocorticoid utilization must be avoided. Hence, the judicial considerable use of glucocorticoids in COVID-19 cases is necessary because this aggravates the hyperglycemia condition and advances the formation of diabetes ketoacidosis. Apart from this, in COVID-19 treatment, there is also an increment of D-Dimer as a product of cross-linked fibrin (inappropriate blood clot or thrombus formation). To treat the inappropriate formation of thrombus, the immunomodulatory drug tocilizumab is using, which also unluckily, promotes the mucormycosis infection.[68] Therefore, the use of drugs like tocilizumab which are targeting the immune pathways is discouraged without any transparent benefit.[25,148] Moreover, the virus causes the dysregulation of the immune system and as a result, using consistent immunomodulatory medical drugs like tocilizumab can further raise this dreadful infection in the patients of COVID-19 disease.[25,148,149] So, it is necessary to use judicially under intense monitoring of the patient to detect an early fungal infestation.[138]

7. Conclusion

The recurrence of mucormycosis, opportunistic microorganisms has

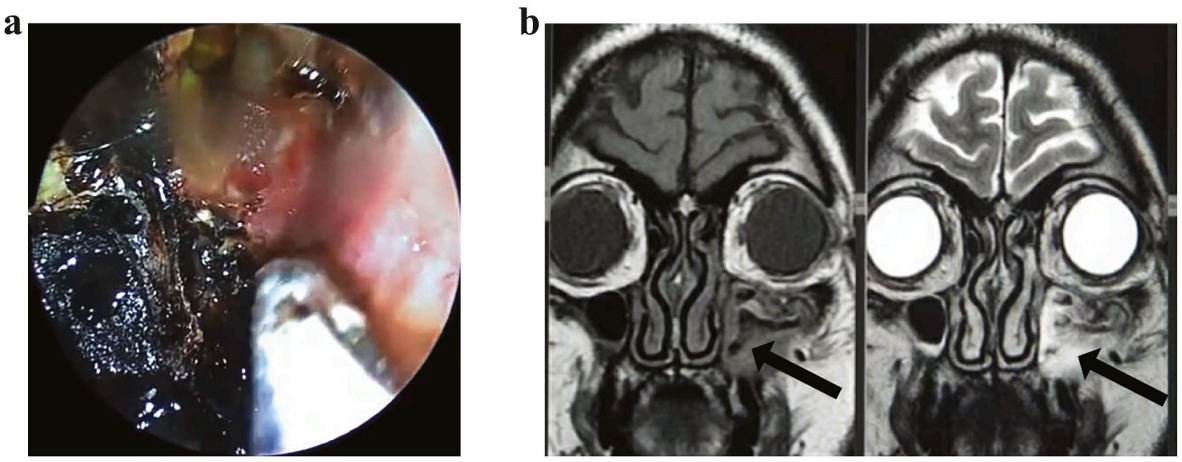

Fig. 7. Illustration of mucormycosis infection spreading to the nasal vestibules, maxillary sinus, and brain. (a) Nasal endoscopic view, showing the location of mucormycosis. **(b)** The magnetic resonance imaging (MRI) scan of the head and sinuses showing the location of pus accumulation and inflammation of the maxillary sinus due to mucormycosis (T1 weighted and T2 weighted MRI scans).

expanded altogether in the previous twenty years. This study gives an overview of comparative cases of different countries, along with the implications of the disease. The rise in mucormycosis emerges to be the result of certain factors including diabetes, uncontrolled use of glucocorticoids (which raises blood glucose, free iron that advances the probable fungal infection), and COVID-19 infection (cytokine storm, neutropenia, endothelial cell surface injury). The involvement between the fungal species of *Rhizopus* and the endothelial cells has also been featured. The mechanism concerning the pathogenesis of the disease has been comprehended and would initiate a vital role in future elevation. Recent tentative regimens for the treatment of mucormycosis comprises the usage of Amphotericin B and Isavuconazole. The administration of therapeutic substances should be closely managed to obtain a therapeutic impact at the moderate possible dose and for the shortest possible duration under keen observation. In the future, an improved establishment of the criteria regarding the diagnosis for COVID-19 associated mucormycosis is required including the radiological patterns of COVID-19 and the difficulty of isolating *Rhizopus* spp. Finally, rapid diagnosis and surgical debridement are considered to be the keystone for this life-threatening disease.

Authors' contributions

Conceptualization: [Joy Sarkar]; Methodology: [Joy Sarkar]; Formal analysis and investigation: [Joy Sarkar]; Writing – original draft preparation: [Deganta Ghosh], [Sagardeep Dey], [Himanko Chakraborty], [Ankita Halder], [Akash Sarkar], [Sneha Mukherjee], [Pallab Chakraborty], [Rajdeep Ghosh]; Writing – review and editing: [Joy Sarkar]; Funding acquisition: [N/A]; Resources: [N/A]; Supervision: [Joy Sarkar].

Declaration of competing interest

On behalf of all listed authors, the corresponding author declares that there is not any sort of financial and non-financial conflict of interest in the subject materials mentioned in this manuscript.

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Review article

Clinical aspects and presumed etiology of multisystem inflammatory syndrome in children (MIS-C): A review

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ABSTRACT

The COVID-19 outbreak sparked by SARS-CoV-2, begat significant rates of malady worldwide, where children with an abnormal post-COVID ailment called the Multisystem Inflammatory Syndrome (MIS-C), were reported by April 2020. Here we have reviewed the clinical characteristics of the pediatric patients and the prognosis currently being utilized. A vivid comparison of MIS-C with other clinical conditions has been done. We have addressed the probable etiology and fundamental machinery of the inflammatory reactions, which drive organ failure. The involvement of androgen receptors portrays the likelihood of asymptomatic illness in children below adolescence, contributing to the concept of antibody-dependent enhancement.

1. Introduction

The COVID-19 pandemic generated by Severe acute respiratory syndrome coronavirus 2 has swiftly expanded globally with about 18 million confirmed reports by August 2020, after a multitude of pneumonia occurrences resulting from unexplained causes was formerly detected in Wuhan (China) in December 2019. Children generally account for a tiny percentage of COVID-19 instances. However, there is confusion regarding the real disease risk of adolescents and children, due to asymptomatic illness, inadequate examination of diagnostically quiet or moderate cases, or doubts about the accuracy of existing testing protocols.[1] In children, COVID-19 hospitalization was uncommon, contributing to only 0.1% of all fatalities.[2] But between April 2020, and July 2020, there has been an upsurge in the incidence of a Kawasaki-like disease in youngsters by 30 times. Pediatricians in the United Kingdom initially declared a group of children having fever, cardiovascular shock, and hyper inflammation in April 2020, with symptoms that were identical to those of Kawasaki Disease, cytokine storm, or toxic shock syndrome on the grounds of clinical studies recorded from United States, United Kingdom, Italy, Switzerland, and France.[3] The ailment was named "pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2" by the Royal College of Pediatrics & Child Health. Next, the Centers for Disease Control and Prevention in the United States and the World Health Organization issued their separate case definitions for the ailment, renaming it as a multisystem inflammatory syndrome in children.[4,5] Scientists have named it profusely like "Kawashocky", "Coronasacki", "hyperinflammatory shock in children with COVID-19″, "pediatric COVID-19″, "Pediatric COVID-19 Associated Inflammatory Disorder" and many more because it's a novel illness. Reports have been identified where, 15 children, 2–15 years old in the

Abbreviations: SARS-COV-2, Severe Acute Respiratory Syndrome Coronavirus 2; KD, Kawasaki Disease; PIMS-TS, Pediatric Inflammatory Multisystem Syndrome Temporally Associated; CDC, Centres for Disease Control and Prevention; PCAID, Pediatric COVID-19 Associated Inflammatory Disorder; MIS-C, Multisystem Inflammatory Syndrome in Children; RT-PCR, Real Time- Polymerase Chain Reaction; PPT, Prolonged Prothrombin Time; LVEF, Left Ventricular Ejection Fraction; ARDS, Acute Respiratory Distress Syndrome; CRP, C-reactive protein; BNP, Brain Natriuretic Peptide; TNP, Tumour Necrosis Factor; TSS, Toxic Shock Syndrome; SHLH/MAS, Secondary Hemophagocytic Lymphohistiocytosis/Macrophage Activation Syndrome; IVIG, Intravenous Immunoglobulin; PTT, The Prothrombin Time Test; ESR, Erythrocyte Sedimentation Rate; ACE2, Angiotensin-Converting Enzyme-2; TMPRSS2, Transmembrane Protease, Serine 2; TTSPs, Type II Transmembrane Serine Protease; AR, Allosomal Androgen Receptor; ADE, Antibody-Dependent Enhancement; NLRP3, NLR family Pyrin Domain Containing 3.

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United States with MIS-C were shifted to the intensive care unit, 102 youths with identical symptoms, were recorded in New York many of whom tested positive for SARS-CoV-2 infection.[3,6,7] 4196 MIS-C Patients and 37 MIS-C deaths, both meeting MIS-C case definitions were confirmed to the CDC by June 28, 2021,.[8] Some organs and tissues, including the heart, lungs, blood vessels, kidneys, digestive system, nervous system, skin, and eyes, become severely inflamed in children who acquire MIS-C. The signs and symptoms vary depending on which body parts are affected.[2,3,9–13] It remains evident whether it is a post-sepsis or delayed infectious consequence or is chiefly connected with SARS-CoV-2 infection, although the recent epidemiologic accounts are extremely provocative of a relationship. [13]

2. Patient demographics

The demographic analysis deals with the assessment of the population, based on variables such as age, race, and sex. Several studies under the present body of knowledge and close monitoring of MIS-C patients have led to a subjective result of the appearance of this syndrome in children.

- Patients with MIS-C had a median age of 9 years. Between the ages of 5 and 13, half of the children with MIS-C were diagnosed. In other studies, the range of age is from 7 months to 20 years, with the highest proportion occurring in youths under the age of 21. [4,8,9,13]
- Early findings showed males may be highly represented, same as KD. MIS-C has yet to demonstrate a definite gender preference, but only a small male preponderance is found in six investigations. Sixty percent of reported patients were male, according to instances reported to the CDC on or before June 28, 2021,.[4,8,14–20]
- Many studies have found that MIS-C has a significant impact on African American, African/Afro-Caribbean, and Hispanic youngsters. African/Afro-Caribbean children constituted the largest fraction of the cases in European research with relevant race/ethnicity data, ranging from 38% to 62% of MIS-C patients. The African American and Hispanic were around 18–40% and 24–45% respectively among the MIS-C affected children, in one of the U.S. reports. And, till June 2020, 62% of all cases confirmed to the CDC consisted of Hispanic children or Latino (1246 cases) or Black, Non-Hispanic (1175 cases).[4,8,10,14,21–25]
- Cases recorded at CDC till June 2020 show that 99% of MIS-C sufferers tested positive for SARS-CoV-2, the rest 1% of patients might have gotten into touch with a COVID-19 infected patient.[8] In a separate US analysis with 577 MIS-C patients, 52% had a positive SARS-CoV-2 Real Time-Polymerase Chain Reaction test result, 45% were solely SARS-CoV-2 antibody positive, 31% were positive for both, and an antibody test was not conducted in 19% of the cases.[10] Out of 29 patients in a finding, SARS-CoV-2 Polymerase Chain Reaction tests yielded positive results in 10 cases, while SARS-CoV-2 immunoglobulin G assays yielded positive results in 19 patients.[26] In these children, the initial COVID-19 infection is nearly often moderate or asymptomatic.[27]
- Feldstein et al. spotted that 73% of MIS-C affected patients were priorly healthy in case reports of 186 individuals.[16] A vast majority of studies found almost no comorbidities. Obesity and a history of asthma have been the most frequent comorbidities in individuals who did possess past medical issues across studies, with autoimmune illness, long-term lung ailment, diabetes, cancer, congenital heart disease, and neurological disorders as fundamental detections.[4,14,15,18,21–23,28]

2.1. Case definition of MIS-C

The WHO has published the case-definition MIS-C, where the following six criteria are to be fulfilled.

1. Age 0–19 years
2. Fever for ≥3 days
3. The clinical indication of the involvement of multiple organ systems (At least 2 of the mentioned manifestations)
   i. Erythema, bilateral non-purulent conjunctivitis, or mucocutaneous or dermatological inflammation signs on mouth, hands, or feet.
   ii. Hypotension or shock
   iii. Cardiac disability, pericardial inflammation, coronary anomalies, or valvulitis (including echocardiographic findings or elevated troponin/brain natriuretic peptide)
   iv. Presence of coagulopathy (prolonged prothrombin time; amplified D-dimer)
   v. Acute gastrointestinal symptoms (diarrhoea, vomiting, or abdominal pain)
4. Inflammation markers that are elevated (namely, erythrocyte sedimentation rate, C-reactive protein, or procalcitonin).
5. Other microbiological causes of inflammation, like bacterial sepsis and staphylococcal/streptococcal toxic shock syndromes are not identified.
6. Reports testing positive for present or past SARS-CoV-2 pathogenesis by RT-PCR, antibody, or antigen test; or interaction with a person infected with COVID-19. [1,4,11–13,27]

CDC also has a separate case definition that focuses on evidence of clinical symptoms involving several organs.[4,27,29]

3. Clinical manifestation

Knowledge revolving around the clinical condition of MIS-C patients is unfolding day by day.[11] As a significant percentage of SARS-CoV-2 infections has escaped diagnosis, the overall population of children residing in the danger for MIS-C is unclear, owing to the possibility of asymptomatic or paucisymptomatic infections.[4] Based on a temporal link of SARS-CoV-2 invasion with MIS-C, the average time between primary infection and the incidence of MIS-C symptoms, in children with a recorded history of confirmed or suspected COVID-19 infection, is two to six weeks.[4,29] The establishment of a severe inflammatory state is one of the major symptoms of MIS-C including, spiking and persistent fever (>39°C-40 °C) with severe asthenia for a few couples of days, myalgia, swollen hands or feet, and multisystem damage (Fig. 1).[3,4,6,9,11,14,27–29]

3.1. Cardiovascular symptoms and image finding

Patients initially felt chest pain, with an average delay of 6 days between the outset of clinical symptoms and the outset of heart failure symptoms. They experienced cardiogenic shock upon their entry to the pediatric intensive care unit and were provided with inotropic support.[11,20,30] All of the investigations found cardiac abnormalities using echocardiography or electrocardiography, highlighting the appearance of myocardial dysfunction.[4] Echocardiography revealed depressed systolic function, with left ventricular ejection fraction of <55% (moderate dysfunction) and sometimes <30% (severe dysfunction),[19,21,30] pericarditis (pericardial effusion) and myocarditis, atrioventricular valve regurgitation, cardiac dysrhythmia, coronary dilation, or aneurysms with a medial z score range of 2.0–2.8 indicating small aneurysm and rarely giant aneurysm were reported.[3,4,10,12–14,21,27,29–31] In adolescents with vasodilatory shock, cardiac magnetic resonance imaging (MRI) revealed signs of myocardial edema, necessitating fluid resuscitation.[13,29] Cardiac involvement is an extensive factor to differentiate MIS-C from COVID-19. [10]

3.2. Respiratory symptoms and image findings

Though COVID-19-like respiratory complaints are not often

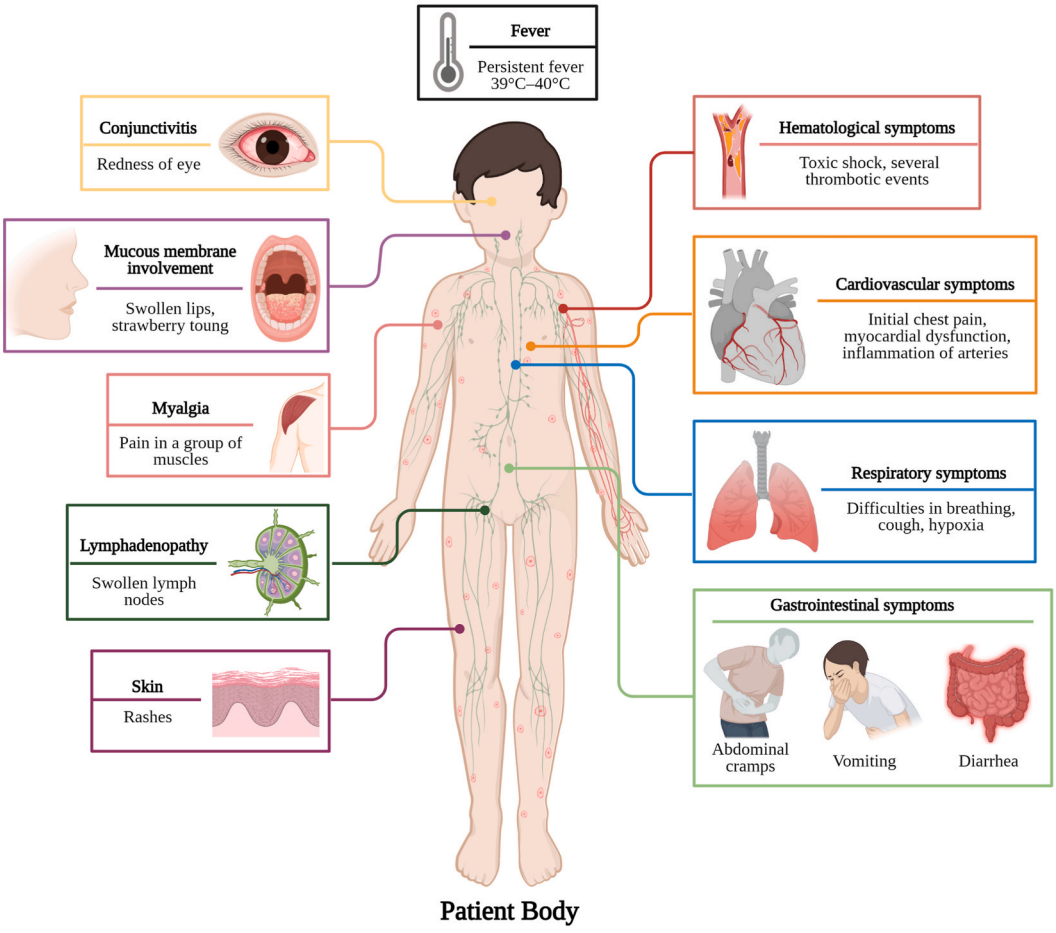

Fig. 1. Various types of MIS-C symptoms in pediatric patients. **(Created with** BioRender.com**).**

associated with MIS-C, difficulties in breathing like tachypnoea, cough, hypoxia, have been disclosed so far. Chest radiographs showed pulmonary edema, basilar opacities suggestive of atelectasis, either dependent or coercive as a consequence of pleural effusion, pulmonary infiltrates, pneumothorax, pulmonary hemorrhage, and bronchospasm, requiring the utility of bronchodilators continuously. Critical pulmonary infection, such as acute respiratory distress syndrome, was uncommon in children who needed supplemented oxygen or a ventilator for breathing support. [4,6,10,11,21,27,29,31,32]

3.3. Neurological symptoms and image findings

The youngsters have been observed with various neurologic issues. Headaches, hearing & visual problems, amnesia, meningitis, irritability, apathy, and lassitude are some of the symptoms. Encephalopathy, stroke or abrupt intracranial hemorrhage, uveitis, coma, seizures, demyelinating disease, aseptic meningoencephalitis (strengthening pro-inflammatory Central nervous system feedback),[31] and brain death were among the profound neurologic findings seen in specific cases. Rare instances reported ischemic brain infarction, acute cerebral edema, and Guillain-Barre syndrome.[4,10,11,20,27,29,31]

3.4. Gastrointestinal symptoms and image finding

Gastrointestinal involvement was usually the most apparent attribute of MIS-C, reported in maximum patients often resembling abdominal infections.[4,11,12] Abdominal cramps, diarrhoea, and vomiting were among the prominent symptoms.[4,11,12,14,27,29] Abdominal ultrasonography and computed tomography of the abdomen and pelvis disclosed grave results like appendicitis, gall bladder hydrops, ascites, mesenteric adenopathy, pleural effusions, enterocolitis, in certain cases terminal ileitis and colitis, all leading to hypovolemia. The pancreatic images reported pancreatomegaly, and those of the liver reported hepatomegaly, and biliary sludge, while increased renal echogenicity, lead to acute kidney failure.[4,9,10,21,29,32]

3.5. Mucocutaneous and dermatological symptoms

The mucocutaneous results were heterogeneous. Morbilliform, urticarial, scarlatiniform, and reticulated forms were among the morphologic features of exanthemas.[26] The area of the skin affected also differed where certain individuals were with restricted acrofacial inclusion while others harbored more extensive outbreaks.[26] Some studies have also revealed a strong age bias in the advent of symptoms.[26] The prevailing cutaneous records were conjunctivitis, hyperemia, periorbital swelling and erythema, and strawberry tongue. A few dermatological findings were whereas malar rashes, facial edema, palmar erythema, lip cracks, and lip hyperemia causing redness and swelling.[4,10,11,21,26,29,32] In a special case, a skin biopsy presented lymphocytic infiltrate as the root of skin lesion.[26]

3.6. Hematological findings

MIS-C patients were found with several thrombotic events where activation of coagulation lead to deep vein thrombosis, intracardiac thrombosis, cerebral venous sinus thrombosis, subarachnoid hemorrhage bringing about ischemic brain death.[10,13,20,27,33] A prothrombotic coagulopathy may be enhanced by MIS-C's hyperinflammatory

condition in conjunction with COVID-19 triggering pulmonary embolism.[6,33] Additional hematologic abnormalities comprise lymphopenia, neutrophilia, haematolysis, hypoxemia, ischemia, anemia, pancytopenia, and hemolytic uremic syndrome.[10,11,33]

3.7. Lymphatic findings

Swollen lymph node often called adenopathy has been noted as a common sign of inflammation in MIS-C-affected children encompassing distinct organs like mesenteric lymphadenitis and mediastinal and hilar lymphadenopathy which have been observed through thoracic imaging.[6,11,32]

3.8. Laboratory findings

The common feature found in every MIS-C patient is an extremely elevated level of inflammatory and cardiac indicators.[4] Inflammatory indicators like C-reactive protein, Serum interleukin-6, Ferritin, Procalcitonin are significantly raised.[4,11,13,27,29,31,34–36] The elevated values of CRP, Ferritin and Procalcitonin vary as 11.98–27.62 mg/dL, 370.7–1032.5 ng/ml and 8.41–31.96 ng/mL respectively.[14] The values of cardiac indicators like Troponin[4] and Brain natriuretic peptide[4,11] vary as 0.03–2.17 ng/mL, 229.5–1778.5 pg/mL respectively.[14] Another characteristic feature of MIS-C is raised levels of D-dimer,[4,13,14,20,30–32,34–36] Fibrinogen,[4,13,14,29] Factor VIII.[13] The value varies as 2.42–3.79 μg/mL for D-dimer[14] and 468.5–629 mg/mL for Fibrinogen.[14] The low percentage of Antithrombin III causes several types of thrombosis in patients.[13] Cytokines like Tumour necrosis factor, Interleukin-6, IL-1β are synthesized in excess amounts, which upregulate the inflammatory reaction.[13,30] MIS-C patients show abnormal Liver function test results having elevated Alanine transaminase and Aspartate transaminase.[32,34,35] The values of ALT and AST vary as 27.73–73.6 U/L and 36.25–56.75 U/L respectively.[14] MIS-C patients show a comparatively lower value of Lactate dehydrogenase enzyme than patients having severe COVID-19. [14] A higher Erythrocyte sedimentation rate value is also very common in MIS-C patients.[11] ESR value varies as 38–58 mm/hr[14] Low blood Sodium[14] and Albumin [29,35,3636] and a high Creatinine[34] value are revealed by laboratory examination in MIS-C patients. According to a recent study, severe COVID-19 instances have a greater neutrophil to lymphocyte ratio.[34]

4. Comparing MIS-C with other associated diseases

Following the past COVID-19 infection, new publications have revealed that MIS-C possesses symptoms of an array of different disorders namely KD that had originated in Japan in 1967, Toxic Shock Syndrome that had originated in 1978, Secondary Hemophagocytic Lymphohistiocytosis/Macrophage Activation Syndrome, and Severe COVID-19 (Table 1).

5. A plausible course of patient management

There exist no definitive therapeutic guidelines for the treatment of MIS-C at this time, but few current administration and therapy options are available. Most of these treatment strategies have yielded a positive result.[4] Intravenous immunoglobulin and corticosteroids have been proven to be effective in various studies as a remedy for inflammation, leading to a quick recovery.[4] Use of IVIG similar to normal KD therapy and corticosteroids[4,13,15,20,30,44] has been encountered in MIS-C patients.[9,11,13,21,26,32,45,46] Patients with a low index of suspicion present with some but not all of the MIS-C symptoms should be examined for inflammatory screening, including a complete blood count and CRP, along with SARS-CoV-2 PCR and antibody testing.[13]

5.1. Hospital treatment

Empiric antibiotic coverage is prescribed in children, who have been assessed for having MIS-C and have been admitted to the hospital, with initial broad-spectrum antibiotics, since symptoms overlap with severe bacterial infections. Ceftriaxone is generally suggested if they are sick to a moderate extent. In cases of severe illness or shock, vancomycin, clindamycin, and cefepime, or vancomycin, meropenem, and gentamicin are recommended.[13,45,47] If redeliver (an antiviral drug with activity against SARS-CoV-2 approved for compassionate use in young children and restricted clinical trials) is available, it must be evaluated, especially for individuals who have been PCR positive and/or have a characteristic COVID-19 presentation.[4,9,13,20,26,45] For children, the current recommended dose is 5 mg/kg IV once (max dose 200 mg) on day 1, then 2.5 mg/kg IV daily for nine days (max dose 100 mg).[13,45] In case of all children exhibiting KD-like illness and evidence of significant inflammation (CRP >30 g/dL, ferritin >700 ng/mL), cardiac involvement, or multi-fold organ failure, 20–25 mg/kg/dose every 6 h (80–100 mg/kg/day) of aspirin is advised as a medication. However, individual health centers may use different amounts of aspirin. When a patient has been afebrile for 24 h or more, the aspirin dose typically reduces to 3–5 mg/kg as a single daily dose, which will be continued after discharge.[9,11,13,30] Anakinra is prescribed at a dose of 2–6 mg/kg/day IV/SQ, with the period of treatment determined with the help of a pediatric rheumatologist or immunologist.[4,11–13,20,21,26,32,48–50] A major percentage of patients got intravenous steroids, Infliximab, and IL-6 inhibitors (Tociluzimab or Siltuximab) as anti-inflammatory therapy.[4,11–13,20,21,45,51,52] Owing to the involvement of TNF-α in MIS-C, anti-TNF-α medication is useful for the control of auto-inflammatory disorders in which many cytokines are high, implying that anti-TNF-α therapy may stop a cytokine cascade on its own.[51,53]

5.2. ICU treatment

A significant percentage of MIS-C patients are referred to the ICU, frequently requiring respiratory and cardiac assistance. Several studies indicated that about 44–100% of the children were sent to the ICU.[30] A major proportion of children also required routine ventilation.[18] Mild to medium doses of vasoactive medicines, like vasopressors and inotropes, were regularly administered to MIS-C ICU patients due to shock-induced by myocardial dysfunction (e.g., acute myocarditis) and/or intense vasoplegia.[22,30]

5.3. Discharge norms

Studies have revealed several guidelines that are to be taken care of before patients are discharged off. Some of them include two days without fever, two days out of vasopressors and supplemented oxygen, two to four days of declining inflammatory markers like ferritin, D-dimer, CRP, lowers levels of troponin, standard Electrocardiogram (the German spelling- Elektrokardiogramm) with stable blood pressure.[4,13,20,45,51] Patients released from the emergency unit must receive particular discharge manuals including a follow-up clinic or telemedicine consultation within 72 h. A repetition of the laboratory tests must be conducted within one week. The interval between the initial echocardiography and the cardiology follow-up should be at least two weeks.[13]

6. Case study

COVID-19 instances (after COVID as well as current COVID) linked to MIS-C have been discovered all over the world. Some of the occurrences from various nations have been summarised in Table 2 and Table 3 simultaneously.

Table 1
Comparison between multisystem inflammatory syndrome in children (MIS-C), Kawasaki disease (KD), toxic shock syndrome (TSS), secondary hemophagocytic lymphohistiocytosis/macrophage activation syndrome (SHLH/MAS), and severe COVID-19.

Sl. No.	Characters	Multisystem inflammatory syndrome in children (MIS-C)	Other diseases associated with MIS-C					References
			Kawasaki disease (KD)	Toxic Shock Syndrome (TSS)	Secondary Hemophagocytic lymphohis-tiocytosis/ Macrophage activation syndrome (SHLH/MAS)	Severe COVID-19 in children without MIS-C	Severe COVID-19 in adults	
1.	Age of affected persons	Children of age range 8–10 are most commonly affected.	Usually in youngsters of less than five years of age.	Usually in children above the age of ten.	Mostly found in adults.	Adolescents are most commonly affected.	Death rates are increasing as people get older.	2,4,10,11,29,35,37
2.	Differences in gender	Males are mostly affected.	Males are mostly affected.	Females are mostly affected.	Occurs in males as well as females	There is no such differentiation. Both the genders are affected equally.	Males are mostly affected.	11,37
3.	Affected Ethnicity	Hispanic/ Latino/African American	East Asian	No ethnic variation known	No difference	No difference	No difference	4,10,11,29,37
4.	Symptoms A. Hypotension	May be present or absent.	Generally absent	Almost always present	Generally absent	May be present or absent.	Almost always present	11,29
	B. Rash	Generally present	Generally present	Generally present	Bleeding from the skin is noted in some cases.	May be present or absent.	May be present or absent.	2,4,38
	C. Fever	Present	Present	Present	Present	Present	Present	4,10,11,35,37–39
	D. Vomiting/ Diarrhoea/ or abdominal pain	Almost always present	Rare	Generally present	May be present or absent.	May be present or absent.	May be present or absent.	11,35,38,39
	E. Respiratory distress	Generally present	Rare	Almost always present	Generally present	Generally present	Generally present	11,37
	F. Mucous Membrane Involvement	May be present or absent.	Generally present	May be present or absent.	Noted in some cases	Generally present	Generally present	11,38
5.	Underlying etiology	Assumed to be a post-infectious syndrome; the SARS-CoV-2 antibody test is frequently positive; in seronegative individuals, there is generally a history of exposure to a covid-19 positive individual.	No identifiable cause.	An infection caused by streptococcus or staphylococcus is a regular occurrence.	T-cells and macrophages possess hemophagocytic activity to expand and become highly activated.	There may be underlying comorbidity; SARS-CoV-2 RT-PCR is generally positive.	SARS-CoV-2 RT-PCR is frequently positive; Extreme sickness is frequently caused by pre-existing comorbidity.	4,29,37,404
6.	T Cells	Lymphopenia	Involvement of cytotoxic T cells	Lymphopenia	Activation and proliferation of $CD8^+$ T cells and NK cells, including secretion of IFNγ	Usually, unaltered	Lymphopenia in severe disease	37,40
7.	Comorbidity as risk factors	Immune deficiency states may be present.	Rarely observed when it comes to original immunodeficiency and occasionally in case of acquired immunodeficiency.	Normally, nothing noteworthy	The cytokine storm plays a role in coronavirus infection. COVID-19-associated pneumonia. Some people have minimal or mild lung manifestations, with others having severe pulmonary dysfunction.	Comorbidity like malignancy, chronic lung disease and neurological disorder is linked to a more severe form of the disease.	Comorbidity like hypertension, diabetes mellitus, chronic heart disease is linked to a more severe form of the disease.	37,40,41

(*continued on next page*)

Table 1 (*continued*)

Sl. No.	Characters	Multisystem inflammatory syndrome in children (MIS-C)	Other diseases associated with MIS-C					References
			Kawasaki disease (KD)	Toxic Shock Syndrome (TSS)	Secondary Hemophagocytic lymphohistiocytosis/ Macrophage activation syndrome (SHLH/MAS)	Severe COVID-19 in children without MIS-C	Severe COVID-19 in adults	
8.	Predominant manifestation	Gastrointestinal signs (abdominal discomfort, diarrhoea) are common, with more than 80% of patients experiencing them.	Symptoms of the gastrointestinal tract are rarely noticeable.	Rash, hypotension.	Unremitting fevers, cytopenia, splenomegaly, hepatitis, coagulopathy, lymphadenitis, and hepatosplenomegaly multisystem organ failure, and death in its most severe form.	Cough, respiratory distress may be present, gastrointestinal symptoms are less common.	Cough, respiratory distress is common.	[37,42,43]
9.	Management	IVIG; steroids; IL-6 inhibitors IL-1 impeders.	IVIG; steroid; IL-1 blockers	Antibiotics, IVIG	Involvement of particular cytokines in this phenomenon, especially TNF-α, IL-6, and IL-1β	Antibiotics, antiviral medication, steroids, IVIG, IL-6 inhibitors	HCQS; steroids; IL-6 inhibitors, plasma in remission; antiviral therapies.	[37,41]

7. The most feasible mechanism of the build-out of MIS-C

Pediatric patients distressed with MIS-C exhibit large amounts of SARS-CoV-2 antibodies in their serum but test negative for the virus through RT-PCR, indicating that certified reports of COVID-19 are relatively few in children or they might have had a prior infection.[1,3] The feedback from antibodies in children was unique from those of the adults stating that the induction of adaptive immune reaction to SARS-CoV-2 virus in the former corresponds with the onset of inflammatory symptoms and is not influenced by viral attack.[1]

The dominant receptor for the ingress of the virus inside the human body is the Angiotensin-Converting Enzyme-2 receptor, which renders its activity along with Transmembrane protease, serine 2 cell surface protein, representing a type II Transmembrane Serine Protease, preferably in the alveolar pneumocytes.[3,37,54–58] Mainly TMPRSS2 sunders the S- protein of SARS-CoV-2 utilizing its protease activity, into two parts S1 and S2, which facilitate binding of the virus and its unification with the target cell respectively (Fig. 2A).[3,37,59,60] The gene encoding for TMPRSS2 protein has been spotted in chromosome 21 of humans, whose transcription is modulated by allosomal androgen receptor transcription factor.[54,55,58,61,62] Sex-steroid hormones such as testosterone reactive promoter sequence existing upstream of the gene, thereby deploy AR's activity through several signaling systems.[54,55] Though hints of estrogen affecting the task of TMPRSS2 have been obtained, nevertheless male sex hormones form the chief regulator of TMPRSS2 bringing about a lower rate of AR activity in females in contrast to males, featuring the varying levels of extremity and mortality due to COVID-19 disease in different genders.[3,54,55,63,64] Alongside lower amounts of androgens in prepubescent children conceals TMPRSS2 activity in their lung cells which contributes to the reduced prevalence and extremity of COVID-19 related inflammation in pediatric patients.[3,41,54,55,65,66] Thereupon, adrenarche is an essential milestone that describes the reason for the greater vulnerability of children 10–12 years or above, to MIS-C, signifying that those children have entered the adrenarche stage that enhances androgen output. This age-dependent revelation of ACE2 and TMPRSS2 eases viral entry in adolescents causing pronounced pathogenesis and MIS-C symptoms, while curbing viral access in pre-adolescents minimizing their symptoms.[3,54,55,67,68]

Moreover, the higher concentration of serum antibodies in pediatric patients portrays the possible operation of antibody-dependent enhancement mechanism[3] in provoking MIS-C, which is more certain to arise as an outcome of acquired immune response and not due to enhanced multiplication of virus.[1,3,69] Certain viral disorders, like dengue and Zika virus infections, have well-documented ADE pathways.[3,69] Reports on MIS-C patients producing neutralizing[70] and non-neutralizing (binding) antibodies[3] as feedback to the spike protein of SARS-CoV-2 have been obtained. Neutralizing antibodies confers sterilizing immunity by negating the pathogenic effect of the virus while the non-neutralizing one attaches to the virus but doesn't possess the potential to nullify its virulence.[3,41,70] It is thought that when children are first exposed to the SARS-CoV-2 virus, their immune system produces both of these antibodies. Later on, the youngsters overridden with neutralizing antibodies are likely to suffer from asymptomatic sickness but, virus attack and critical multisystem inflammation are boosted in them with prevalent binding antibodies via ADE.[3] Non-neutralizing antibodies or inadequate quantities of neutralizing antibodies bound to the epitopes of SARS-CoV-2, in the patient's blood, promote its intake inside the host tissue which is described as ADE. This machinery is unassociated with the ACE2 pathway and involves uniting of the complex of virus epitope and virus-specific non-neutralizing antibody by dint of the F_C domain of immunoglobulin to the immune cell's membrane harboring IgG Fc receptor (F_cR).[3,71] This interaction activates macrophages, natural killer cells, lymphocytes, and monocytes causing cellular endocytosis[3,41] (Fig. 2B). Endocytic Toll-like receptors such as TLR3, TLR7 detect the viral RNA and thus make the macrophages operational, inducing a surge of pro-inflammatory cytokines like TNF-α, IL-6, IL-18, IL-16, IL-1β occasionally by the NF-κβ route.[3,37,41,71–73] This originates a cytokine storm mimicking the provocation of macrophages as seen in hemophagocytic lymphohistiocytosis.[3,74] $CD68^+$, CD169+ macrophages[3] aid in viral dispersion and induce pyroptosis via inflammation[41,75] (Fig. 2A). Pyroptosis indicates cell death linked to the NLR family pyrin domain containing 3 inflammasome activation systems. The cellular damage thus instigates the surrounding macrophages to generate chemokines and cytokines[3] furthermore indicators of inflammation can also help macrophages to engage T-cells in the infection area.[41,72,76,77] Elevated levels of IL-1β in blood serum evince the occurrence of pyroptosis.[41] A probable role of non-specific antibodies[3] has been put forward that justifies the genesis of MIS-C through ADE, in seropositive patients where non-specific antibodies unite with the virus aiding its intake by the immune cells. Gruber et al. (2020)[78] have also studied the role of auto-antibodies found against endothelial and gastrointestinal cells in MIS-C patients, which fails to distinguish

Table 2
Table showing the case studies of the individual patients having Post COVID-19 MIS-C.

Sl. No.	Region	Schedule of patient admission	Patient's description	Symptoms and image findings	Laboratory findings	Similarities with	Treatment	Reference
1.	Atlanta, Georgia	June 2020	A 25-year-old woman	Exhaustion, dyspnea, mild cough and low-grade fevers, vomiting, sore throat, diarrhoea, slight hypotension (blood pressure 98/56 mmHg) and usual blood oxygen level in indoor air, cervical lymphadenopathy; notable conjunctival injection; red and cracked lips; left lower abdominal tremble. Echocardiogram: Enlarged inferior vena cava, cardiac dysfunction. CT of abdomen/pelvis: Stranding of peri-pancreatic fat; pancreatitis, indefinite bilateral perinephric fat stranding.	Troponin-I was discovered at 0.06 ng/mL; high levels of creatinine (7.74 mg/dL), BNP (378 pg/ml), d-dimer (960 ng/ml), ferritin (798 ng/ml).	KD	To minimize the chances of thromboembolic and nephrotoxicity, IVIG (2 g/kg) was administered in uniform dosages on the second and third day of the hospital admission, along with aspirin (325 mg) for 7 days, and redeliver.	[9]
2.	New York	May 2020	An 11-year-old female	Initial: sore throat, uneasiness, low appetite, leg and abdominal pain, pruritus skin rash, fever (39.3 °C), tachycardia (126beats/minute), hypotension, slight dehydration, erythematous palm with a widespread reticular, non-blanching papular rash across the belly and bilateral upper extremities. Echocardiogram: Decreased systolic function of the left ventricle. Electrocardiogram: Showed sinus tachycardia and S1Q3T3 indicating strain on the right side of the heart.	Uplifted levels of troponin (0.112 ng/mL) and BNP (8718 pg/mL). White blood cell count increased to 14.18 causing lymphopenia. PTT yielded an increased value of 1.9 along with the raised levels of IL-6 (0.0–15.5 pg/mL), ferritin (13.00–150.00 ng/mL), D-dimer (0–243 ng/mL, procalcitonin (0.00–0.50 ng/mL), CRP (0.10–2.80 mg/L) and normal level of creatinine (0.53–0.79 mg/dL).	TSS, septic shock, cytokine storm, KD, SHLH	Furosemide along with antibiotics like clindamycin, ceftaroline, and piperacillin-tazobactam was administered. Enoxaparin was started as a comprehensive anticoagulant. Vitamin K was employed to improve elevated INR and PT. An IL-6 blocker, tocilizumab, was progressed along with convalescent plasma therapy and remdesivir.	[45]
3.	Not found	Not found	A 14-year-old boy	Fever, tachycardia and inflamed maculopapular rash on the face, abdominal sensitivity, as well as a perianal injurydischarging pus. A 28-cm ileitis, a 2.3-cm perianal pustule, and a fistula were diagnosed on magnetic resonance enterography.	Initially, tests revealed a normal ESR rate (0–5 mg/L) and normal levels of CRP (0–15 mm/h). Increased serum amounts of IL-6 (73.4 pg/mL), IL-8 (21.8 pg/mL), IL-1β (0.4 pg/mL), TNF-α (97.8 pg/mL) were noticed in the cytokine profile up to eight days of hospitalization, which later on declined till the tenth day on treatment with infliximab.	KD	Azithromycin and hydroxychloroquine were used for SARS-CoV-2 infection, intravenous piperacillin/tazobactam was used to cure perianal abscess, and enoxaparin was utilized for the prevention of venous thromboembolism, along with intravenous fluid therapy.	[51]
4.	Kerala, India	April 2020	A 5-year-old boy	Fever of high intensity, abdominal cramps and watery stools, pyuria, bulbar conjunctivitis without pus and non-pitting edema of the feet and hands, tachycardia (130 beats per min), vasoplegia. Echocardiogram: Comprehensive left ventricular hypokinesia with medium systolic dysfunction (EF = 35%) and myocarditis was discovered. Chest X-ray: Disclosed cardiomegaly.	Inflammatory cytokines in blood serum-like CRP, ferritin, creatinine and liver enzymes were found to be upraised. The results of a complete blood count revealed neutrophilic leucocytosis.	KD	Pulmonary support using a high flow nasal cannula with a 2 L/kg flow rate was attempted, as well as inotropic support was provided with adrenaline; Ceftriaxone, an injectable antibiotic, immunoglobulins, diuretic drugs, enalapril and methylprednisolone pulse (30 mg/kg/d for 3 d), were some of the remedies.	[47]

between self and non-self cells, ultimately attacking a patient's native tissues.[1,78] Thus, it can be inferred that localized inflammation and the build-up of pathogenic macrophage congregations in body tissues are two especially common factors that cause MIS-C syndrome and more analysis is needed to illustrate the role of macrophages further.[3]

Table 3
Table showing the case studies of the cohorts having Post COVID-19 MIS-C.

Study	Region	Schedule of patient admission	Description of patients (number, age/ interquartile range [IQR])	Number of Patients detected positive	Symptoms and Image findings	Laboratory findings	Similarities with	Treatment	Reference
Trevor K. Young et al. (2020)	New York	April 1 to July 14, 2020	A cohort of a patient (total = 56) IQR = 0.7–17 years	PCR: 10/56 IgG Tests: 19/56 Mucocutaneousfindings: 27/56	Fever for 1–2 days, mild cough. Major mucocutaneous findings (in 21) included strawberry tongue (in 8), lip crack (in 13), conjunctivitis (in 21), erythmateous hands and feets (in 13), cheek (in 6) and orbit of the eye (in7). Eruptions of several types, i.e., mobiliform (in 3), reticulate (in 3), scarletiniform (in 5) and urtecarial (in 5). Gastrointestinal and cardiac trouble.	D-dimer, BNP, and troponin levels were all enhanced.	KD	Injectable immunoglobulin, corticosteroids, Aspirin, Remdesivir, Anakinra.	[26]
Blumfield*et al.* (2020)	New York	April 21-May 22, 2020	A cohort of patients (total = 16) IQR = 20 months to 20 years.	RT-PCR: 3/16 IgG Tests: 10/16 Both RT-PCR and IgG Tests: 1/16	Fever (in 16), erythema (in 10), emesis (in 12), diarrhoea (in 7), abdominal discomfort (in 11), conjunctivitis (in 8), headache (in 6), and hoarseness (in 5) were the first symptoms, followed by breathing issues and congestion (in 1), hypotension (in 10), and ischemia (in 7). Echocardiography: Systolic myocardial abnormality (in 10), ectatic coronary arteries (in 4), and pericardial effusion (in 2) were discovered on echocardiography. Chest radiography: Megalocardia (in 10), cardiogenic pulmonary edema (in 9), and a modest pleural discharge (in 7) were seen on chest radiographs, with only a few patients developing pneumonia (in 1) and acute respiratory distress syndrome (in 2). CT scan of abdomen and pelvis: Abdominal fluid build-up (in 6), hepatomegaly (in 6), mesenteric lymphadenitis (in 2), and thickening of the urinary (in 1) and gall bladder (in 3) walls were all seen on abdominal radiography.	Erythrocyte sedimentation rate (in 12), CRP (in16), D-dimer (in 16), troponin (in six), and pro-BNP (in 15) values were all raised. High white blood cell count (in 13) leading to leucocytosis and hypoalbuminaemia (in 16) were encountered too.	Kawasaki Disease (KD)	Intravenous corticosteroids Intravenous immunoglobulin and Anakinra	[32]
Belhadjer et al. (2020)	France and Switzerland	March 22 to April 30, 2020.	A cohort of a patient (total = 35) IQR = 2–16 years.	Nasopharyngeal swab PCR: 12/35 Fecal PCR: 2/35 Antibody Tests: 30/35	All of the children had a fever and weakness, and 80% of them had gastrointestinal issues (in 29) such as abdominalache, diarrhoea, and vomiting. Runny	Heightened IL- 6, D-dimer, troponin, CRP and BNP.	KD	Inotropic support, Immunoglobulin infusion, Intravenous corticosteroids, IL-1 inhibitor and therapeutic dose of heparin.	[28]

(*continued on next page*)

Table 3 (*continued*)

Study	Region	Schedule of patient admission	Description of patients (number, age/ interquartile range [IQR])	Number of Patients detected positive	Symptoms and Image findings	Laboratory findings	Similarities with	Treatment	Reference
					nose (in 15), skin rashes (in 20), meningism (in 11), angina (in 6) mesenteric and cervical lymphadenopathy (in 21) were among the additional symptomatology. Echocardiography: It denoted impaired left ventricular systolic activity, with an EF of 30–50%, resulting in left ventricular hypokinesia (EF<45%) in 31 individuals.				
Whittekar E. et al. (2020)	England	March 23 to May 16, 2020.	A cohort of a patient (total = 58) IQR = 3 months-17 years.	PCR: 15/58 IgG Test: 40/58	Every single patient had a continuous fever for 3–19 days, as well as a variety of conditions such as pharyngitis (in 6), headache (in 15), abdomen ache (in 31) and lymphadenitis (in 9). Manifestations of the mucosa included distended hands and feet (in 9), erythema (in 30), conjunctival injection (in 26), reddish cracked lips (in 17). They also exhibited renal injury (in 13) and cardiac shock (in 27). Echocardiography: Malfunctioning of the left ventricle.	All of the patients exhibited a significant inflammatory response in terms of elevated levels of CRP, troponin, ferritin, N-terminal pro-BNP and neutrophilia.	PIMS-TS and Kawasaki Disease (KD) shock syndrome.	Intravenous immunoglobulin (in 41), Corticosteroids (in 37), Anakinra (in 3) and Infliximab (in 8).	[21]
Kaushik et al. (2020)	New York	April 23 to May 23, 2020	A cohort of a patient (total = 33) IQR = 6–13 years.	RT-PCR: 11/33 Antibody Test: 27/33 Both test: 6/33	Major portion of the patients had fever (Avg. temperature of about 39.4C°) (in 31) and other symptoms like uneasiness of the stomach/vomiting (in 23), diarrhoea (in 16), dyspnoea (in 11), vertigo (in 3), low blood pressure (in 21), peritoneal pain (in 21), mucocutaneous involvement (in 7) like conjunctivitis (in 12) and dermatological symptoms like rashes (in 14), and also neurological involvement (in 4). Echocardiogram: Depressed LVEF with various range of EF was observed (in 21). Chest Radiograph: Megacardia (in 10) and in addition bilateral pulmonary opacities were noted (in 11).	Inflammatory indicators like CRP, procalcitonin, D-dimer, ferritin, ESR, and fibrinogen were found to be increased. There were also heightened markers of aberrant cardiac state like, troponin, N-proBNP, and BNP.	Toxic shock	Intravenous immunoglobulin (in 18), Corticosteroids (in 17), Tocilizumab (in 12), Remdesivir (in 7), Anakinra (in 4), Convalescent plasma therapy (in1), Norepinephrine (in 10) and Dopamine (in 9).	[3,4]

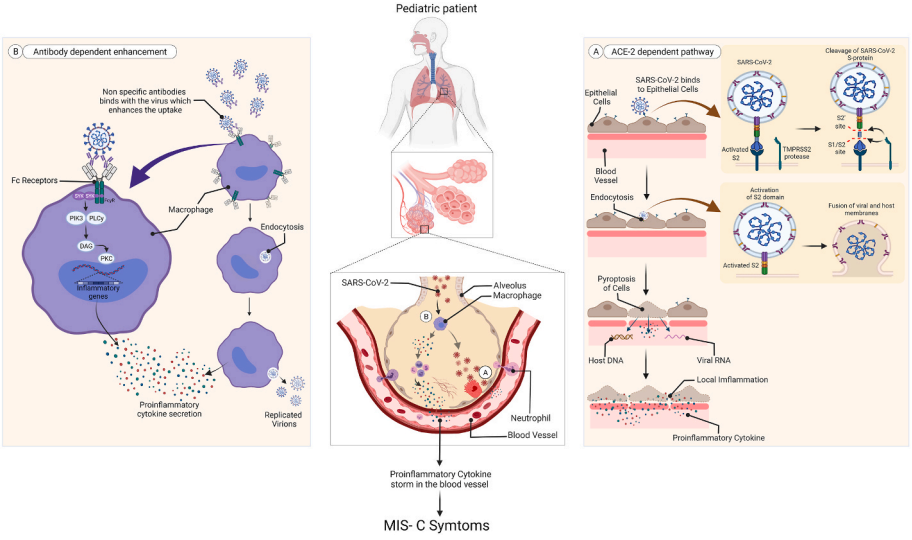

Fig. 2. The most likely mechanism for MIS-C expansion in pediatric patients.[1,3] **A.** ACE-2 dependent pathway **B.** Antibody-dependent enhancement. **(Created with** BioRender.com**).**
[ACE-2 = Angiotensin-converting enzyme 2. DAG = diacylglycerol. FcyR = Fc-gamma receptor. MIS-C = multisystem inflammatory syndrome in children. PIK3 = phosphoinositide 3 kinase. PKC = protein kinase C. PLC-y = phospholipase C gamma. SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2. SYK = tyrosine protein kinase SYK. TMPRS52 = transmembrane serine protease 2.].

8. Conclusion

MIS-C is generally curable and rarely happens, but a certain lack of knowledge could make it severe in the long term aspect.[4] As it is a rare condition, most children who have it improve with medical treatment. However, some children swiftly deteriorate to the point where their lives are jeopardized. As the number of MIS-C cases related to COVID-19 is increasing incessantly, it can be clearly stated that COVID-19 is not only just a respiratory disease,[6] further elaborate research is needed to know more about the etiology of MIS-C associated with COVID-19, as it is still unknown how the risk factor for MIS-C varies among child community.[4] Children develop COVID-19, unlike adults, by ADE due to a lack of androgens,[3,54] which directly regulates the TMPRSS2 receptor.[55,66] Therefore, to prevent the transmission of COVID-19 in this age group, parents should be more careful of their children in surroundings with a high population density.[4] Precautions and safety measures such as social distancing, use of face masks, frequent washing of hands, use of alcohol-based disinfectants, should be followed in places like schools,[4] parks, crèche, etc. Parents, babysitters, teachers, and school officials should primarily be cognizant of the indications and signs of both COVID-19 and MIS-C so that proper treatment is provided before its late.

Authors' contributions

Conceptualization: [Joy Sarkar, Suchismita Kumar]; Formal analysis and investigation: [Anusrita Kundu, Joy Sarkar]; Writing – original draft preparation: [Anusrita Kundu], [Swagata Maji], [Suchismita Kumar], [Shreya Bhattacharya], [Pallab Chakraborty]; Image Preparation: [Pallab Chakraborty]; Writing – review and editing: [Joy Sarkar]; Funding acquisition: [N/A]; Resources: [N/A]; Supervision: [Joy Sarkar].

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Consent to participate

All the authors mutually agree to participate in this work.

Consent for publication

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Declaration of competing interest

On behalf of all listed authors, the corresponding author declares that there is not any sort of financial and non-financial conflict of interest in the subject materials mentioned in this manuscript.

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Review

Comparative review on left-handed Z-DNA

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1. Abstract

Being polymorphic, deoxyribonucleic acid is worthy of raise a variety of structure like right-handed B to left-handed Z conformation. In left-handed contour of DNA consecutive nucleotides substitute between syn-arrangement and anti-arrangement, through the chain. 2D gel electrophoresis comprising d(PCpG)n of topo isomers of a plasmid inserts d(pCpG)n, in this 'n' ranges among 8 to 21, indicate the change of B-Z DNA. The high denseness of salt is required for conversion of B configuration d(CG)n toward Z configuration. The rate of B to Z transition is measured by "Cytosine Analogues" and "Fluorescence Spectroscopy". h-ZαADAR1 that a Z-DNA's binding domain, binds and stabilizes one part in Z configuration and therefore the remaining half in B deoxyribonucleic acid configuration. At halfway point, it creates B-Z junction. "Stacking" is the main reason for the B-Z DNA junction construction. Upregulation of ADAM-12, related with Z-DNA is said to a cause for cancer, arthritis, and hypertrophy. Z-DNA forming sequence (ZFS) conjointly generates massive - scale deletion in cells from mammals.

2. Introduction

In 1979, a left-handed crystal deoxyribonucleic acid structure was published, which convey a unique zigzag, sugar-phosphate backbone, it's named as Z conformation of deoxyribonucleic acid (Z-DNA) and it's all biological relevance had yet to be established [1, 2]. It was already known that normal right-handed B conformation can assume a diverse number of configurations, under certain torsional stress [3]. Z configuration exists in high energy state than the common B-DNA configuration. This conformation has negative super helicity which soothes the structure. In contrast to B form with anti-conformation, in Z-DNA convey anti-conformation and syn-conformations alternately by rotating around glycosyl bonds, along with the chain [4]. Under bound condition non-B-DNA structure like cruciform, triplex, hairpin, etc. are formed by collapsible monotonous DNA sequence. This unusual structure has effects on several biological progressions [5]. Super helicity is the most significant inducer for Z contour in usual DNA. Non-super helical, natural DNA holds practically no Z-DNA, but other hand the same DNA under extreme negative super helicity, as in "form V" may have as much as 35–40% of its sequence in Z arrangement [6]. Except for Z-DNA, X-ray fiber diffraction outlines were framed and

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Table 1. Comparable information between A-DNA, B-DNA, Z-DNA [2, 9].

Parameter	A-DNA	B-DNA	Z-DNA
Helix sense	Right-handed	Right-handed	Left-handed
Axial raise [in Armstrong]	2.55	3.4	3.7
Helix pitch	28°	34°	35°
Base pair tilt	20°	-6°	7°
Rotation per residues	33°	36°	-30°
Diameter of helix [in angstrom]	23	20	18
Glycosidic bond configuration	Anti	Anti	Anti
da, dT, dC, dG	Anti	Anti	Syn
Inserted phosphate phosphate distance [in Armstrong]	5.9	7.0	7.0
da, dT, dC, dG	5.9	7.0	5.9
Suger pucker	C3'-endo	C2'-endo	C2'-endo
da, dT, dC, dG	C3'-endo	C2'-endo	C3'-endo

differentiates several conformations of DNA. Most DNA enters the A-DNA conformation which's per turn contain 11 bp through right-handed helix [7]. The single-crystal method resolute the complementary structure, oligo deoxy nucleosides, d(GGTATACG) and d(IODO-CCGG) [7, 8] (Table 1).

Existence of B-Z transition and Z-DNA is further deep-rooted by the specific ZBP discovery [10]. *In vitro*, Z-DNA was postulated for identification of proteins that bind with it in a structure-precise manner, act as a cis-element and aid in biological development. RNA Double Strand adenosine deaminase 1 is a type of the ZBP [11]. This ADAR1 has a Zα domain capable of transform B into Z conformation and create the junction [12, 13]. Formation of Z-DNA is induced by a unique sequence motif. Sometimes, it presents frequently adjacent with the start site of transcription and induce the transcription [14–16]. The junction between B-Z is formed with the help of ZBP. Formation of this portion carries out flipping over of bases, stacking of bases, and infringement of one base pair [17]. In another study also verified that normal B form also transfers into Z form by elevation of salt of aggregation [18, 19]. In humans, Z-DNA first came into consideration through the autoimmune disease Lupus erythematosus [20]. Z-DNA formation sequence (ZFS) is found to be associated with immune retorts and infection genome uncertainty. The Z configuration is also evidenced to be linked with large scale deletion in the cells of mammals [21, 22]. It also controls the genes transcription regulation of c-myc and CRH of human [23, 24].

3. Z-DNA structure

The optical investigation originally proposed the Z-DNA. The result of the experiment exhibited that a 4 mL NaCl solution contains a polymer which consists of discontinuous cytosine and guanine residue and formed a nearly inverted circular dichroism gamut [25]. Until 1979, the invention of Z-DNA remained unknown. Original atomic steadfastness exposed that it was not the same right-handed B-DNA which was invented by James D. Watson & H.C. Crick in 1953. Despite that, this new left-handed helical structure named as Z deoxyribonucleic acid. This Z form consists of extremely immunogenic antibodies to recognize the configuration, unlike B form of DNA [26]. There have some familiar features of B form with the d$(Cg)_3$ system. The antiparallel double-helical structure holds Watson-Crick base pairing between the base of Guanine and Cytosine. The left-handed helicity oligomers have six base pairs with significant regularity. Balance correlated hexamers stack on one other so closely in an endless polymer of alternating cytosine guanine residues sequence [2].

Various conformational topographies differ the Z-DNA from the B-DNA (Fig. 1). The double-helical Hexanucleoside Penta Phosphate molecules allied with the crystal. Crystal of Z arrangement contains discontinuous cytosine and guanine residues'-DNA is dinucleotide while B-DNA is mononucleotide with anti-configuration. All deoxycytidine has anti-configuration whereas all the deoxyguanosine has anti syn-configuration.

In Z arrangement the base pair is lifted from the center, so the guanine imidazole ring is originated at the edge, but in case of B-DNA those bases are at the center. In B configuration 34Å pitch with 10.5 bp is present where Z configuration convey 44.6Å pitch with 12 bp per turn [9]. Six levels of base pairing have been seen in the d$(Cg)_3$ structure because of C1 base pair with G12, G2, C11 and so on. Z-DNA is not slanted with each other straight, but they remain linked to a literal translation of 7Å relative to each other so that it can shear the appearance from one another with a little rotation throughout the chain. Despite being stacked on other bases the guanine is loaded upon the oxygen atoms of prior deoxyribose residues. The backbone of sugar-phosphate is constant for both the Z form and B form. In B configuration the minor-grooves are above the base pairs. But in Z form minor-grooves exist below the base pair [2].

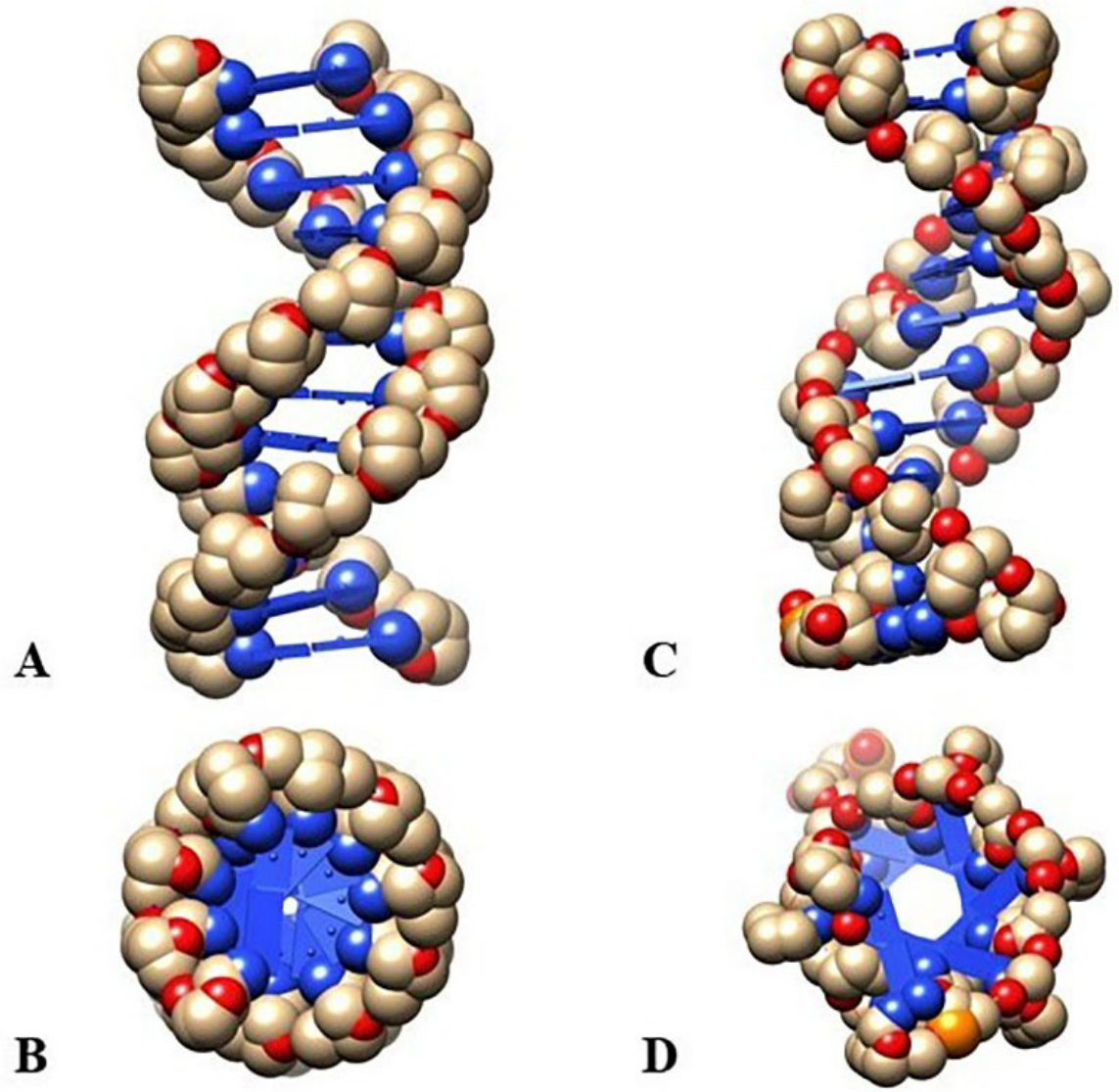

Fig. 1. Evaluation of the structure between Z and B-DNA configuration. (A, B) Z-DNA configuration showing left-handed elongated spiral with a lateral view and a polar view. (C, D) B-DNA configuration showing right-handed elongated spiral with a lateral view and a polar view.

4. B-DNA to Z-DNA transition

Earliest Harvey model is used for examining B-Z transition. This model defines the procedure which is engrossed by another longitudinal DNA conscious models. Base pairs opening was another early observed mechanism regarding this model before the Wang model. At the present portrait of Zipper Model demonstrated that Z-DNA contains high energy joint that grows through the DNA polymer until the full B-Z trans polymer gets transferred into Z-DNA. Though there are some problems in the model because it does not disclose many vibrant structural details, so it has limited applications in thermodynamics. There are several facts regarding the transition of B-Z such as the high concentration of salt in the solution which balance the Z-DNA due to massive reduction in electrostatic repulsion in the pillar of phosphate. Negative helicity of deoxyribonucleic acid needs energy that can also uncoil B form to configure the Z form. Z-DNA can also be stabilized by transcription.

Maruyama and colleague establish the B-DNA to Z-DNA transition communed by a method called “cationic graft copolymer” where the Poly (L-lysine)-graft dextran (PLL-g-Dex), begins with two-step method including the creation of a clear intermediate [27]. Amid DNA phosphate group electrostatic repulsion reduce by the cationic backbone of the copolymer and the transition is a result of these 2 factors. The most plausible Z form created negative supercoiling, utilizing B-DNA occurs during several metabolisms like Transcription and replication processes [28]. For reducing the transition stress, unusual such DNA as Z-DNA is formed [6, 29]. Lee *et al.* (1992) used “Magnetic-tweezers” and FRET combinedly to examine at molecule level of negative supercoiling [30]. Mag-

netic tweezers are a very useful technique for investigating wind/unwinding procedure of twisted DNA through precisely controlling infinite tension [28, 31]. Therefore, B-Z change can be active by tiny negative super helicity and approximately one Pico Newton Tension. This outcome suggests that in tension Z arrangement is formed more easily *in vivo* [32]. Methanol, Ethanol, Ethylene Glycol (Dehydrating agent) balance the Z-DNA configuration. Due to adjacent clustering counteractions all over the DNA, though more strong ionic properties, thus it provides additional mutually repelling phosphate groups [6]. Antibodies and ZBP can bind the Z form of DNA selectively. This conformation has triggering capability. The Qu group had been reported that Alzheimer amyloid protein brings about the Z-B transition. Forming the Z-form is correlated with Alzheimer's disease [33, 34]. Bae *et al*. analyzed to transition from B-Z conformational change occurs by Z-DNA binding protein unravel the detailed binding machinery and whether the protein industriously initiates Z-DNA's or passively traps transitionally performed Z form. Therefore, it proved that the conformational selection mechanism stabilized the Z-DNAs by alternating the "induced fit" mechanism. A chemical modification also stabilizes Z-DNA transformation [4]. Bulky group's introduction precise in a certain base also steady the growth of Z arrangement by increasing static hindrance.

5. B-DNA and Z-DNA hybrid junction

Double-stranded adenosine deaminase RNA is an enzyme of the deaminase family which edited the appearance of the ds-mRNA by converting adenosine to inosine and creating diversity between RNA and Protein [11]. It is noted as a naturally stirring protein with obvious specificity for methylated and hemi-brominated DNA contains discontinuous deoxy guanosine-deoxycytidine residues [13]. ADAR1 carry two binding motifs for Z-DNA, Zα and Zβ [11].

A few numbers of investigations were completed to show the interface between the solution of DNA and Zα ADAR1 domain. If the DNA solution is interacting with dodecamer (d(CG)$_6$) it produces the B-DNA circular-dichroism spectrum. When Zα ADAR1 is mixed into the solution the spectrum progressively altered, which mirrored Z conformation. This demonstrated that the Zα domain is equipped for alleviating the dodecamer in the Z configuration. Brownian motion or Pedesis is the reason for this twist of dodecamer fragment. After this conformational change, DNA binds with the Zα domain to prevent the reappearance of B-DNA conformation [12, 13].

Kim *et al.* in 2005 developed a DNA duplex with 15 bp and with two hanging nucleotides [17]. This DNA duplex is co-crystallized with the ZαADAR1 domain (amino acids 140-202). So, Z-DNA is tightly bound with the binding domain of Z DNA, h-ZαADAR1. After the binding, it stabilizes one half in the Z configuration and remaining part in B form. In the centre portion, a B-Z junction is created [17]. At this DNA duplex, eight bases stabilized with normal Z-DNA conformation [2]. The remaining six bases are maintaining the typical B conformation [35]. On the link point, A-T bases are disrupted from each other and make a sharp turn, which obliged an inversion in the way of the backbone. This creates a bent at the intersection point of B-Z DNA. The disrupted A, T bases adopted anti-conformation. Base A is extended out from the helix and T is slanted analogous to the spiral. But first base-pair from the Z-DNA after the junction creates a long rise distance which clearly showed the stacked A-T the bases within the B-DNA conformation. Stacking is the main stabilizing factor for the junction, and it is proved that one bp extruding by breaking can cause reversion of the handedness of the duplex. Other than A-T bases, it is equally possible for other G-C bases to be extruded [17]. Thermodynamic examinations of the melting of oligomers holding the junction show that the edifice of the hybrid junction from B-DNA declines the melting free energy by 0.5 kcal/mol [36]. This B-Z configurational change and syn-conformation of both bases are done by base 'flipping over'. A torsional strain breaks and causes base extrusion. This extruded base is allowed to flip over and reorganization the bp, which creates a ZIP-like movement in two direction. This movement for the limitation of the ZFS with an extruded base at the intersection. Base-pair disruption, expulsion, and reconstruction are lengthening the Z-DNA segment through an additional negative torsional strain of chromatin [17].

Another investigation also proved that B-Z DNA junction can be produced by oligomeric sequences in the aqueous solution at 3 M or high salt concentration. The 5.5 M NaCl with a 95 mM combination induces the A-T sequence into the Z-DNA conformation [18]. This study re-establishes that when $NiCl_2$ is added in the salt solution, it creates a striking change in Raman Spectra, indicating A-T bases are adopting the Z conformation [19].

6. Z-DNA in human disease

In living body, Z-DNA can form and role as a dynamic component in various genome's metabolic courses under certain biological circumstances [21]. Z-DNA is used in many precise activators or repressors enrolment for directive gene countenance, genome uncertainty control [22]. Another study proved that in cells of mammal's ZFS fetch genetic uncertainty. Repair mechanism can proceed with the Z-DNA development in the mammal's body, which creates a large genomic alteration. These sorts of changes are relevant to the breakage and translocation near ZFS in human lymphoma and leukaemia [9]. In humans, Z-DNA links with the transcription of the c-myc genes, which means when the Z-DNA development is turned off the cell gives a signal as a result, c-myc transcrip-

tion also starts to down-regulate [23]. In the same way, Z-DNA development is also associated with the corticotropin-releasing hormone (CRH) gene transcription [37]. On the other hand, the human body also shows the activation of the Nrf2 gene which is relevant to the HO-1 gene's promoter, which allied with Z-DNA development [24]. A few numbers of immunoglobulin-related genes (example-ETV6) are enriched by the Z-DNA sequence. But in blood cancer, these genes are related to translocation of the chromosome [22]. Interferonopathies disease like Aicardi-Goutières Syndrome is caused by Mutation, which reduces p150 Z-binding with impaired enzymatic activity. This is induced by dsRNAs and most commonly these dsRNAs derive from Alu retroelement. The Z-DNA and Z-RNA both are essential for limiting Alu retroelement intrusion of primate genomes [38]. Z-DNA provides a base for therapeutically reducing the chances of Arthritis, Cancer, and cardiac hypertrophy. This role is believed to be arbitrated by the downregulation of ADAM-12. It was observed that ADAM-12 protein expression is raised when there are pieces evidence of arthritis, cancer, and cardiac hypertrophy. Whereas ADAM-12 expression level Is exceptionally low in certain adult tissue. The regulation of ADAM-12 is related to the highly conserved region containing a stretch of dinucleotide repeat sequence and known as negative regulatory element (NRE), which serves as a repressor of ADAM-12 expression. There is a certain Z-DNA binding protein-like MeCP2. It modulates the ADAM-12 repression by recruiting NF1 transcriptional factors. Loss of ZFS leads to a low level of MeCP2 which results in metastatic breast cancer [22, 39]. Apart from this, HIF1α induced Z-DNA development in the microsatellite of slc11a1 gene promoter. It was also perceived to control its definite allele expression in patients of rheumatoid arthritis, tuberculosis [40]. Z-DNA also has an immunogenic character and it can prevent systemic lupus erythematosus. But in the patient's sera of these diseases, some anti-Z-DNA antibody are found. Two kinds of antibody are found, first-one responsible for denaturation of both B and Z form and second-one is Z-DNA specific [20, 41]. Z arrangement also induce conformation instability by acting as a site for cancer-related genes like scl, bcl2, and c-myc [9]. B-Z junction is a site where CAG trinucleotide repeat instability happened. X fragile chromosome and skeletal dysplasia associated with CGG repeats and GAC trinucleotides repeat respectively [42–44]. In a study, typical left-handed Z-DNA was originated in brains of severe AD affected patients. Similarly, the moderately affected patients showed the existence of B-Z intermediate conformation in their brain DNA. Immunohistochemical data has proved that the total amount of Z form is one-seventh than B arrangements in human's genome [45, 46]. It was also observed that some genes, related to Alzheimer's like presenilin-1, presenilin-2, APOE (Apolipoprotein E), etc. are overexpressed in patients and has an important appeal in Alzheimer's pathogenesis. Z-DNA existing in the brains of Alzheimer's patients are far more vulnerable to hydroxyl radical-induced damage of DNA, in comparison to A-DNAs or B-DNAs. This was due to the occurrence of more exposed bases and patients with severe Alzheimer's showed the existence of both Z-DNA and damaged DNA of similar types [47]. This finding has again been confirmed from another study which showed that Z-DNA became sensitive to hydrolytic enzyme DNase I, on incubation with Aβ protein for a certain period [34]. This results in alteration of Z arrangement back into normal B form. These transition of Z form to normal B form is verified as quicker process when an interaction of Aβ is made, in the existence of ethylene glycol also [48].

7. Conclusions

Z-DNA is a double-helical structure that preserves antiparallel backbone of sugar-phosphate chains with Watson Crick pairing. Despite that, it has a contour which is fundamentally dissimilar from B configuration of DNA. Two-dimensional Gel Electrophoresis offers us a powerful method to examine the super helicity-induced physical revolution in the DNA. Besides this, B-Z conversion is also designated here. One of a reasons for transition is a cause of free unfavourable energy. Affected advances are unrestricted from the uniting effect of genomics, human genetics, biophysics, and molecular studies on non-B-DNA configurations through mutation causing agents, intricate in Genetic diseases. Autoimmune processes may be suspected in all clinical conditions where specific anti-Z-DNA antibodies are found, but for further investigation, larger population is wanted to prove such an immunological hypothesis. Future prominence will challenge to tune the acceptance of the non-B-DNA configurations at a definite location of genes to correlate this behavior extra thoroughly with the generation reposition terminuses. Also, the analysis to recognize the kind of non-B-DNA structures that obtain certain sort of mutations and the fascinated enzyme on the evolution of therapeutics, to ameliorate the disturbing corollaries of these disorders.

8. Author contributions

PC and RR conceptualize this review article. RR analyzed and interpreted the information regarding Z-DNA structure and B-Z DNA transition. PC performed a study on B-Z DNA hybrid junction formation and effects of Z-DNA on human disease and was a major contributor in writing the manuscript. AC developed the figure based on available data. PC prepared the final draft of the manuscript under the supervision of JS. All authors read and approved the final manuscript.

9. Ethics approval and consent to participate

The work reported here in the manuscript is original and free from any plagiarism. All the data in the article are real and authentic. All the co-authors have read and agree to publish all the items listed above.

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Reetabrita Roy and Pallab Chakraborty contributed equally to this article.

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12. Conflict of interest

The authors declare no conflict of interest.

13. References

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Abbreviations: Aβ protein, Amyloid β-protein; AD, Alzheimer's disease; ADAM, a disintegrin and metalloproteinase; ADAR1, Adenosine Deaminase Acting On RNA; APOE, apolipoprotein E; CRH, corticotrophin-releasing hormone; DNA, Deoxyribonucleic acid; FRET, fluorescence resonance energy transfer; HO-1, heme oxygenase-1; IODO, 3-Iodo-L-tyrosine; MeCP2, methyl CpG binding protein 2; mRNA, messenger RNA; NF1, neurofibromatosis type 1; NRE, negative regulatory element; PLL-g-Dex, Poly (L-lysine) - graft dextran; RNA, Ribonucleic acid; ZBP, Z-DNA binding protein; ZFS, Z-DNA forming sequence.

Keywords: Alzheimer's disease; Wang model; Z-DNA-binding protein; Z-DNA-forming sequence; Review

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Green synthesis of copper/copper oxide nanoparticles and their applications: a review

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REVIEW

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Green synthesis of copper/copper oxide nanoparticles and their applications: a review

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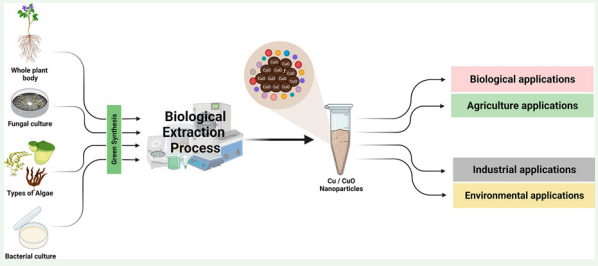
ABSTRACT

Copper and Copper oxide nanoparticles have garnered a lot of attention among the metal oxide nanoparticles, especially because of their many characteristics and applications in many disciplines, notably nanomedicine and biomedical sciences. We have covered all of the conceivable green production techniques of copper/copper oxide nanoparticles in this review. This manuscript also diagrammatically depicts the exact mechanism of all conceivable biosynthetic routes. We also look at the antibacterial, antifungal, antiviral, and anticancer properties of biosynthesized copper/copper oxide nanoparticles, as well as their effects on plant growth, nutrition, and defense mechanism.



Abbreviations: Cu/CuO NPs: Copper/Copper Oxide nanoparticles; DLS: Dynamic Light Scattering; EDS: Energy Dispersive X-ray Spectroscopy; EDX: Energy dispersive X-Ray; FFT: Fast Fourier transform; FTIR: Fourier transform infrared spectroscopy; HR-TEM: High-resolution transmission electron microscopy, HeLa: Henrietta Lacks; IR: infrared; LSV: Linear sweep voltammetry; NTA: Nanoparticle tracking analysis; PPE: Personal protective equipment; PSA: Particle size analyzer; SEM: Scanning electron microscope; SPR: Surface plasmon resonance; TEM: Transmission electron microscopy; VEGF: Vascular endothelial growth factor; XRD: X-ray diffraction.

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1. Introduction

In the sphere of research, nanotechnology is a relatively new approach. This technology is now widely used (*1*) in diversified fields. The smaller dimension of nanomaterials, ranges from 1–100 nanometers (nm) (*1*), alters their physicochemical properties like shape, size, and chemical composition. In the twenty-first century, a more in-depth investigation of metallic NPs was carried out by several researchers (*2*).

Bionanotechnology is a rapidly growing field of nanotechnology in which bio-organisms are extensively used to synthesize nanomaterials and the synthesized nanomaterials are simultaneously used to improve the quality of life of the organisms (*2*). Biological synthesis uses the biological principle of oxidation and reduction by microbial enzymes or plant phytochemicals (*3*). In recent times physical and chemical methods are mainly used for the synthesis of inorganic NPs (*2*). Both physical and chemical methods have some disadvantages like low-productivity, non-eco-friendly, toxic, and capital intensive. For these reasons, biological synthesis is trying to replace the chemical methods of producing NPs (*4–23*).

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Numerous reports and studies have demonstrated that this green synthesis process has already manufactured a large number of metal/metal oxide nanoparticles such as silver (Ag), gold (Au), selenium (Se), platinum (Pt), zinc oxide (ZnO) and iron oxide (Fe_2O_3), graphene oxide etc. (*4–22*). Additionally, those studies reported that several metal nanoparticles have a variety of biological and biochemical activities, but CuNPs have recently gained attention. Copper plays a variety of roles in humans, including serving as a cofactor for numerous enzymes involved in neuropeptide production, cell signaling pathway regulation, antioxidant defence, and immune cell function (*24, 25*). Copper is needed by plants for a variety of metabolic and physicochemical processes. It's one of the most crucial trace elements for plant growth (*26*). It is present in both humans and plants in a very small amount and helps to regulate different metabolic and biological activities as it acts as cofactors for different enzymes. It is responsible for the normal functioning of different essential proteins/enzymes such as amino oxidase, cytochrome c oxidase, and plastocyanin since it is a cofactor for multiple enzymes (*27, 28*). Copper oxide, on the other hand, has antimicrobial, antibacterial, antifungal, antimicrobial, antifungal, magnetic phase change, gas sensing, biocidal, superconductive, catalytic, and optical properties (*29*). CuO has a bandgap of 1.7 eV and is a p-type semiconductor. The application of biologically synthesized copper nanoparticles (CuNPs) was found to be a promising bioactive agent in this context.

CuONPs are made by different physicochemical methods like sol–gel technique (*30*), sonochemical (*31*), electrochemical method (*32*), microwave irradiations (*33*), solid-state reaction method (*34*), alkoxide based route (*35*) etc. Likewise, these nanoparticles are also generated by the algal, fungal, plant and other biosynthesis routes. In this review, we have focused on almost all the biosynthetic routes of CuNPs/CuONPs. The detailed mechanism of all the possible biosynthetic routes is diagrammatically represented in this manuscript. On the other hand, high catalytic and chemical reactivity, large surface area, and ability to interact with microbe's cells are some of the attributes of CuNPs/CuONPs which enables their application in different fields like agricultural, biomedical, textile, and environmental sectors (*36*). Our review also focuses on the potential applications of biosynthesized CuNPs/CuONPs.

2. Green synthesis of nanoparticles

Green synthesis can be defined as the derivation of materials from green or eco-friendly resources by the use of solvent, good reducing agent, and harmless material for stabilization (*37*). Additionally, this synthesis route is straightforward, cost-effective, dependable, sustainable, and relatively repeatable, and results in more stable compounds. Thus, researchers have expressed an interest in developing a variety of nanomaterials via this biosynthesis route, including metal/metal oxide nanoparticles, hybrid materials, and bioinspired materials. As a result, green synthesis is widely regarded as a necessary tool for mitigating the negative consequences of conventional nanoparticle synthesis methods used in laboratories and industries (*38*). In that context, traditional nanoparticle producing techniques like chemical and physical synthesis are found to be costly, hazardous, and unfriendly to the environment (*39*). Not only that chemical synthesis of nano-production may sometime affect biological activities by some factors like size distribution, morphology, surface charge, surface chemistry, capping agents, etc. (*40, 41*). To avoid the harmful effect, researchers have discovered the precise green pathways, or naturally occurring sources and their products, that may be utilized to synthesize nanoparticles, to address these issues. To circumvent these negative consequences, researchers have identified the precise green pathways, or naturally occurring sources and their products, that can be employed to manufacture nanoparticles.

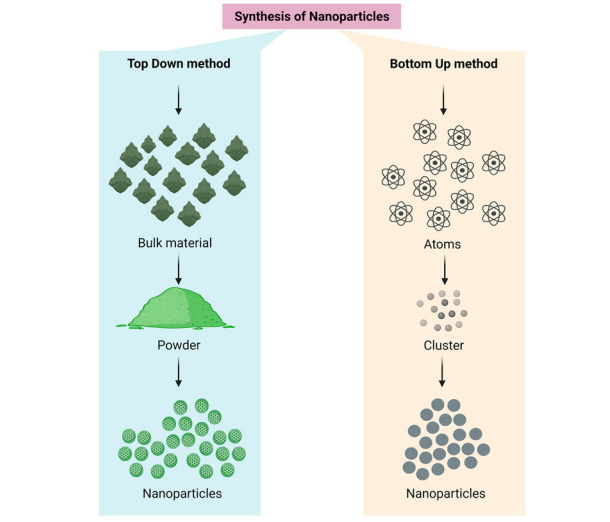
Nanoparticle synthesis can be divided into two categories (Figure 1): (i) Top down method and (ii) bottom up method. The physical route of nanoparticle synthesis is emphasized in the top down method, whereas the chemical and biological methods are emphasized in the bottom up method.

Physical synthesis route consists of pulsed laser ablation, arc discharge, spray pyrolysis, ball milling, vapor and gas phase, pulsed wire dischare, lithography etc. On the other hand, chemical synthesis route consists of chemical reduction, sonochemical, micoemulsion, photochemical, electrochemical, pyrolysis, microwave, solvothermal, coprecipitation etc.

Additionally, green nanoparticle synthesis can be divided into the following categories (Figure 1):

(a) Phyto routes like utilization of plants and plant extracts
(b) Microbial routes like the utilization of microorganisms such as fungi, yeasts (eukaryotes), bacteria, and actinomycetes
(c) Bio-template routes like the utilization of membranes, viruses, and diatoms as templates

Both extracellular and intracellular biological approaches have been employed to synthesize nanoparticles. Although the precise method for the creation of



Synthesis of Nanoparticles		
Top Down method	Bottom Up methods	
Physical process (Toxic)	Chemical process (Toxic)	Green process (Non-toxic)
1. Pulsed laser ablation 2. Arc discharge 3. Spray pyrolysis 4. Ball milling 5. Vapour and Gas phase 6. Pulsed Wire Discharge 7. Lithography	1. Chemical reduction 2. Sonochemical 3. Microemulsion 4. Photochemical 5. Electrochemical 6. Pyrolysis 7. Microwave 8. Solvothermal 9. Coprecipitation	1. Using plants and there extracts 2. Using bacteria 3. Using fungi and yeast 4. Using Algae 5. Using other microorganisms 6. Using enzyme and biomolecules

Figure 1. Various routes of nanoparticles synthesis (Created with BioRender.com).

nanoparticles utilizing biological agents has yet to be discovered, it is suggested that distinct biomolecules are involved for nanoparticle synthesis. Furthermore, the methods for intracellular and extracellular nanoparticle formation differ, and it appears that the cell wall of microorganisms plays a key role in intracellular nanoparticle synthesis whereas extracellular enzymes play a key role in extracellular nanoparticle synthesis. Extracellular nanoparticle synthesis has surpassed intracellular nanoparticle synthesis in popularity due to its faster production rate and simpler synthesis procedure (*42*).

2.1. Probable mechanism of biosynthesis of Cu/CuO-NPs

Cu ions come in a variety of oxidation values, including Cu(I), Cu(II), and a few Cu(III) ions. In terms of plant extract, fungal extract, algal extract, bacterial extract, precursor concentration, pH, and temperature, the synthesis technique reported for CuO, Cu_2O, and Cu_4O_3 is the same to date. These variables, however, have the greatest impact on the type of Cu particles generated during green synthesis (*43*). During the green synthesis, the biomolecules present in the sample extract reduce the Cu^{2+} ion to Cu^0 state and simultaneously oxidize it to form CuO nanoparticles. Certain biomolecules found in the sample extract serve as a capping agent and also aid in the stabilization of the produced nanoparticles (*43*).

2.2. Green synthesis of Cu/CuO-NPs by plant extracts

The environmentally accepted 'green chemistry' idea has been applied to the biosynthesis of nanoparticles for the creation of clean and environmentally friendly nanoparticles, which incorporates bacteria, fungi, plants, actinomycetes, and other organisms, and is referred to as 'green synthesis'. Biosynthesis of nanoparticles utilizing the organisms mentioned above exemplifies a green alternative for the creation of nanoparticles with novel characteristics. Unicellular and multicellular organisms are permitted to respond in these syntheses.

Plants are renowned as nature's chemical factories since they are low-cost and low maintenance. Because extremely minute quantities of these heavy metals are hazardous even at very low concentrations, plants have shown exceptional potential in heavy metal detoxification as well as accumulation, through which environmental contaminants may be overcome (*44–50*). Nanoparticle synthesis using plant extract has benefits over other biological synthesis methods, such as microorganisms, because the rate of metal nanoparticle synthesis with the help of plant extract is more persistent (*51*), significantly faster (*52, 53*), and extremely mono-dispersive (*54*) in respect to other biological methods (*55*). The main challenges for using microorganisms include the toxicity of certain bacteria, the isolation procedure of microorganisms, and the tedious incubation procedure which make them unsuitable for many researchers. Plant extracts are therefore a remarkable source of synthesis of metal and metal oxide nanoparticles (*55–57*). Additionally, the reaction kinetics of plant-assisted nanoparticle synthesis is much faster than other biosynthetic methods that are comparable to chemical nanoparticle production. Plant components such as fruit, leaf, stem, and root have been frequently employed for the green route of nanoparticle production due to the high-quality phytochemicals they generate.

Here, for the mentioned reason, Copper oxide nanoparticles have been widely synthesized using various plant extracts (*58–60*). In this plant-based manufacturing process, the metal salt is mixed with the plant extracts, and the reaction takes 1–3 h to complete at room temperature (Figure 2). Plant extracts include a variety of bioactive metabolites, including flavonoids, phenols, proteins, terpenoids, and tannins, which serve as

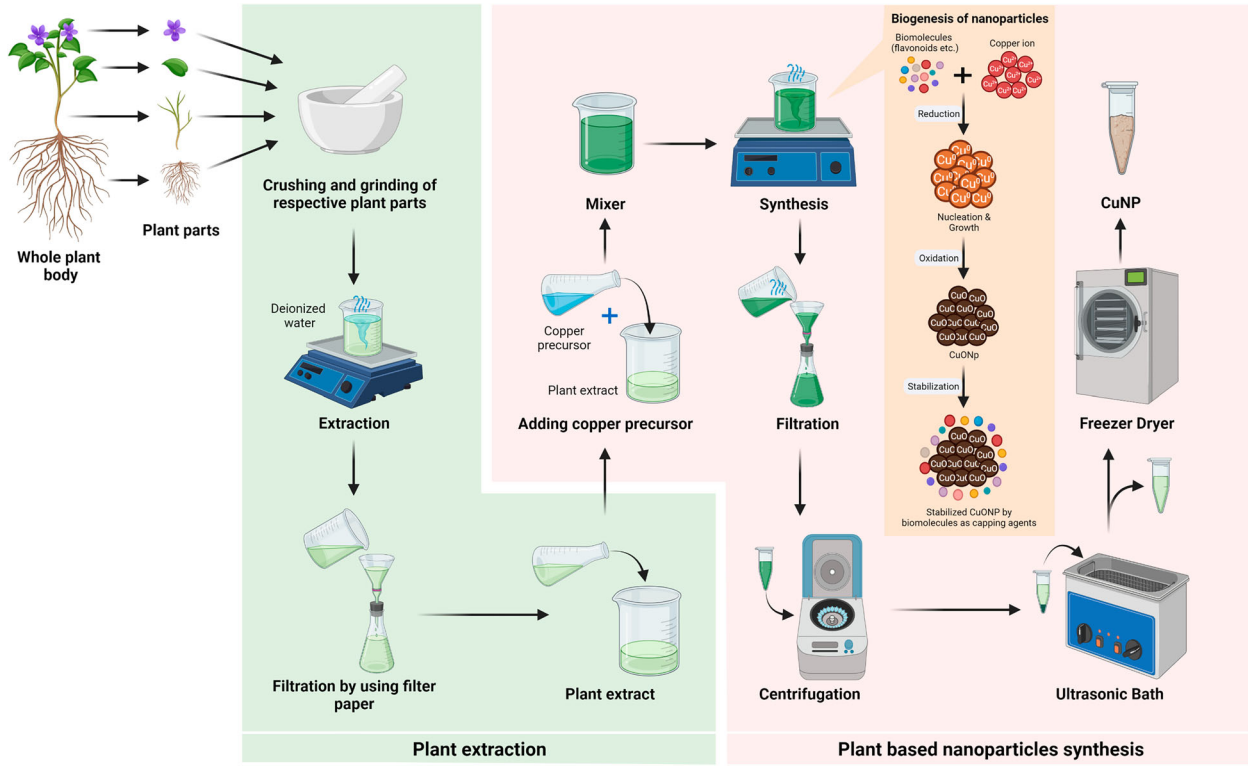


Figure 2. Graphical representation of biomediated synthesis of copper oxide nanoparticles using plant extract (Created with BioRender.com).

reducing and stabilizing agents, transforming metallic ions into nanoparticles (*38*, *44*, *61*). The plant extract produces electrons, which cause copper salts to get reduced. Copper oxide nanoparticles are formed when phytochemicals react with copper ions, resulting in reduction. Table 1 summarizes the major contributions of biomediated synthesis of copper oxide nanoparticles using various plants.

2.3. Green synthesis of Cu/CuO-NPs by using bacteria

Bacteria have been used to make a variety of nanoparticles in recent years, including copper oxide nanoparticles (*127*). Different materials with fascinating shapes and nanoscale dimensions have been produced using bacteria via an intracellular or extracellular route (Figure 3). Bacteria have a great potential for nanoparticles production. They offer benefits such as a short generation period, ease of culture, benign experimental conditions, excellent stability, extracellular nanoparticle synthesis, and ease of genetic modification (*37*). It is known that when microorganisms are maintained in a hazardous metal environment, they develop a method to live by converting poisonous metal ions into non-toxic forms such as metal sulfide/oxides. It has been well established that when bacteria are introduced to an environment containing high levels of hazardous metals, they can survive by converting harmful metal ions to non-toxic metal oxides (*128–131*). Bacteria have been shown to generate a variety of essential thiol-containing chemicals in response to oxidative stress. These molecules function as a capping agent in the bacterially driven production of nanoparticles, preventing metal oxide nanoparticles from oxidizing (*128*, *132*, *133*). The mechanism behind the nanoscale change isn't fully understood to date. Nanoparticle production also requires moderate experimental parameters such as pH, temperature, simple downstream processing, and a short creation period (*134*). Some of the contributions of biomediated synthesis of copper oxide nanoparticles using different bacteria are shown in Table 2.

2.4. Green synthesis of Cu/CuO-NPs by using fungi

Various fungal species have been utilized to synthesize copper oxide and other metal nanoparticles in recent years (*127*). Fungi, as compared to other microbes, have a lot of potential for nanoparticle production. In comparison to bacteria, fungi tolerate agitation, flow pressure, and other conditions in the bioreactor or any

Table 1. Biosynthesis of Cu/CuO nanoparticles by different plants.

Sl. No.	Plants/ plant extract	Precursor	Part used	Size of NPs (in nm)	Shape / structure / morphology	Characterization techniques used	Reference
1.	*Carica papaya* L.	Copper Chloride ($CuCl_2$)	Leaves	20 (in avg.)	Nearly spherical and Crystalline	UV-Vis, XRD, FTIR, SEM, and TEM	(*62*)
2.	*Citrus medica* L.	Copper (II) Sulfate Pentahydrate ($CuSO_4.5H_2O$)	Fruit	20 (in avg.)	Crystalline	UV-Vis, NTA and XRD	(*63*)
3.	*Euphorbia esula* L.	Copper Sulfate ($CuSO_4$)	Leaves	20–110	Spherical	UV-Visible spectroscopy, XRD, FTIR	(*64*)
4.	*Pistacia* sp.	Cupric Chloride Dihydrate	Leaves	9 (in avg.)	Crystallized nanowires with 10 nm diameter	XRD, FTIR and SEM	(*65*)
5.	*Nerium oleander* L.	Copper Sulfate ($CuSO_4$)	Leaves	21	Spherical	UV-Vis and FTIR	(*66, 67*)
6.	*Syzygium aromaticum* (L.) Merr. & L.M.Perry	Copper Sulfate ($CuSO_4$)	Flower	14–50	Spherical and granular nature	UV-visible spectroscopy, XRD, TEM and SEM	(*66, 68*)
7.	*Ocimum sanctum* L.	Copper (II) Sulfate Pentahydrate ($CuSO_4.5H_2O$)	Leaves	77 (in avg.)	Spherical	XRD and FTIR	(*66*)
8.	*Ocimum tenuiflorum* L.	Copper (II) Sulfate Pentahydrate ($CuSO_4.5H_2O$)	Leaves	72	Hexagonal wurtzite crystal structure	UV-visible spectroscopy, XRD, FTIR, SEM and EDAX	(*66*)
9.	*Citrus limon* (L.) Osbeck	Copper Chloride ($CuCl_2$)	Fruits	60–100	Nearly spherical	UV-Vis, FTIR, XRD, SEM and TEM	(*69, 70*)
10.	*Malva sylvestris* L.	Copper Chloride Dihydrate ($CuCl_2.2H_2O$)	Leaves	14 (in avg.)	Spherical	XRD, FTIR and SEM	(*66*)
11.	*Albizia lebbeck* (L.) Benth.	Copper Sulfate ($CuSO_4$)	Leaves	100 (in avg.)	Roughly spherical	UV-Vis, SEM, TEM, EDS and XRD	(*66*)
12.	*Datura metel* L.	Copper (II) Sulfate Pentahydrate ($CuSO_4.5H_2O$)	Leaves	15–20	Spherical	UV-Vis, PSA, TEM, EDX and FTIR	(*71*)
13.	*Magnolia kobus* DC.	Copper (II) Sulfate Pentahydrate ($CuSO_4.5H_2O$)	Leaves	37–110	Spherical	UV-Vis, ICP, EDS, XPS, and HR-TEM	(*72*)
14.	*Ginkgo biloba* L.	Cupric Chloride Dihydrate ($CuCl_2.2H_2O$)	Leaves	15–20	Spherical	UV-Vis, TEM, EDS, and FTIR	(*73*)
15.	*Artabotrys odoratissimus* R.Br.	Copper (II) Sulfate Pentahydrate ($CuSO_4.5H_2O$)	Leaves	135 (in avg.)	Spherical	PSA	(*74*)
16.	*Capparis zeylanica* L.	Copper Sulfate ($CuSO_4$)	Leaves	50–100	Cubical structure	UV-Vis, FTIR, XRD, SEM, EDX and TEM	(*75*)
17.	*Aloe vera* (L.) Burm.f.	Copper (II) Acetate Dihydrate ($Cu(CH_3COOH).2H_2O$)	Flowers	40 (in avg.)	Spherical	UV-Vis, FE-SEM, and FTIR	(*76*)
18.	*Cinnamum* sp.	Copper Sulfate ($CuSO_4$)	Leaves, flowers and roots	18.17-91.28	Spherical	UV-Vis and DLS	(*77*)
19.	*Inula helenium*	Copper Sulfate ($CuSO_4$)	Leaves, flowers and roots	32.41	Spherical	UV-Vis and DLS	(*77*)
20.	*Matricaria chamomilla L.* (Vernacular name: Chamomile)	Copper Sulfate ($CuSO_4$)	Leaves, flowers and roots	58.77	Spherical	UV-Vis and DLS	(*77*)
21.	*Urtica* sp.	Copper Sulfate ($CuSO_4$)	Leaves, flowers and roots	6.5	Spherical	UV-Vis and DLS	(*77*)
22.	*Glycyrrhiza glabra* L.	Copper Sulfate ($CuSO_4$)	Leaves, flowers and roots	28.21	Spherical	UV-Vis and DLS	(*77*)
23.	*Schizandra chinensis* (Turcz.) Baill.	Copper Sulfate ($CuSO_4$)	Leaves, flowers and roots	32	Spherical	UV-Vis and DLS	(*77*)
24.	*Eucalyptus* sp.	Copper Sulfate ($CuSO_4$)	Leaves	38.62 (in avg.)	Spherical	UV-Vis, FTIR, XRD and SEM	(*78*)
25.	*Zingiber officinale* Roscoe	Copper Sulfate ($CuSO_4$)	Rhizome	25–40	Spherical	UV-Vis and XRD	(*79*)
26.	*Eupatorium glandulosum* Michx.	Cupric Nitrate	Leaves	55.91 (in avg.)	Spherical	UV-Vis, FTIR, PSA, TEM and AFM.	(*66*)
27.	*Psidium guajava* L.	Cupric Chloride Dihydrate ($CuCl_2.2H_2O$)	Leaves	13.13 ± 0.19	Spherical	UV-Vis	(*66*)
28.	*Glycine max* (L.) Merr.	Copper Sulfate ($CuSO_4$)	Seeds	20 (in avg.)	Spherical	UV-Vis, TEM and DLS	(*66*)
29.	*Bacopa monnieri* (L.) Wettst.	Copper (II) Sulfate Pentahydrate ($CuSO_4.5H_2O$)	Leaves	20–50	Spherical	UV-Vis, FTIR and HR-TEM	(*80*)

(*Continued*)

Table 1. Continued.

Sl. No.	Plants/ plant extract	Precursor	Part used	Size of NPs (in nm)	Shape / structure / morphology	Characterization techniques used	Reference
30.	*Ocimum basilicum* L.	Copper (II) Sulfate Pentahydrate ($CuSO_4.5H_2O$)	Leaves	40–60	Spherical	UV-Vis, FTIR and HR-TEM	(*80*)
31.	*Asparagus adscendens* Roxb.	Copper (II) Sulfate Pentahydrate ($CuSO_4.5H_2O$)	Leaves	10–15	Spherical	UV-Vis, FTIR and HR-TEM	(*80*)
32.	*Withania somnifera* (L.) Dunal	Copper (II) Sulfate Pentahydrate ($CuSO_4.5H_2O$)	Leaves	50–60	Spherical	UV-Vis, FTIR and HR-TEM	(*80*)
33.	*Phyllanthus emblica* L.	Copper Sulfate ($CuSO_4$)	Fruits	15–30	Spherical	UV-Vis, FTIR, XRD, SEM, EDAX	
34.	*Sterculia urens* Roxb. (Vernacular name: Gum karaya)	Cupric Chloride Dihydrate	Gum	4.8 ± l.6	Spherical	XRD, FTIR, TEM, SEM and XPS	(*66*)
35.	*Gymnema sylvestre* (Retz.) R.Br. ex Sm.	Copper Sulfate ($CuSO_4$)	Leaves	65–302	Spherical	UV-Vis, FTIR and SEM	(*81*)
36.	*Ixora coccinea* L.	Copper Sulfate ($CuSO_4$)	Leaves	80–110	Spherical	XRD, FTIR, SEM, TEM,	(*82*)
37.	*Moringa oleifera* Lam.	Copper Sulfate ($CuSO_4$)	Leaves	6–61	Spherical	XRD, FTIR, SEM, TEM,	(*83*)
38.	*Tridax procumbens* (L.) L.	Copper Sulfate ($CuSO_4$)	Leaves	16 (in avg.)	Spherical	UV-Vis, XRD, FTIR, SEM, TEM	(*84*)
39.	*Camellia sinensis* (L.) Kuntze	Copper Sulfate ($CuSO_4$)	Leaves	50–100	Spherical	UV-Vis, FTIR, XRD, SEM,	(*85*)
40.	*Juglans regia* L.	Copper Sulfate ($CuSO_4$)	Leaves	80 (in avg.)	Spherical	UV-Vis, FTIR, XRD, SEM,	(*86*)
41.	*Acalypha indica* L.	Copper Sulfate ($CuSO_4$)	Leaves	26–30	Spherical	UV-Vis, FTIR, XRD, SEM, EDX, and TEM	(*87*)
42.	*Punica granatum* L.	Copper Aacetate Monohydrate [$Cu(CH_3COO)_2.H_2O$]	Peel extract	40 (in avg.)	Spherical	UV-Vis, XRD, FTIR, SEM	(*88*)
43.	*Musa acuminata* Colla	Copper Nitrate Trihydrate Solution ($Cu(NO_3)_2.3H_2O$)	Peel extract	60 (in avg.)	Spherical	XRD, EDX, FE-SEM, FTIR	(*89*)
44.	*Cordia sebestena* L.	Copper (II) Nitrate Trihydrate Solution ($Cu(NO_3)_2.3H_2O$)	Flowers	20–40	Spherical	FESEM-EDX, XRD, FTIR, SEM, TEM	(*90*)
45.	*Hibiscus rosa-sinensis* L.	Copper Acetate Solution ($Cu(CH_3COO)_2.H_2O$)	Flowers	26.54	Spherical	UV-Vis, XRD, FTIR, and SEM	(*91*)
46.	*Caesalpinia pulcherrima* (L.) Sw.	Copper (II) Nitrate ($Cu(NO_3)_2.XH_2O$)	Flowers	20 (in avg.)	Spherical	UV-Vis, FTIR, XRD, EDAX and SEM.	(*92*)
47.	*Rheum palmatum* L.	Copper Chloride ($CuCl_2$)	Roots	30 (in avg.)	Spherical	UV-Vis, EDX, XRD, FTIR, SEM, TEM	(*93*)
48.	*Desmodium gangeticum* (L.) DC.	Copper Sulfate ($CuSO_4$)	Roots	12 (in avg.)	Spherical	UV-Vis, TGA, XRD, FTIR, TEM, and SEM	(*94*)
49.	*Phaseolus vulgaris* L.	Copper Sulfate ($CuSO_4$)	Whole plant	26.6 (in avg.)	Spherical	XRD, Raman, FTIR, TEM, XPS, DLS, SEM, SAED, and EDX	(*95*)
50.	*Coffea arabica* L.	Copper Sulfate ($CuSO_4$)	Whole plant	262 (in avg.)	Crystalline	UV-Vis, FTIR, XRD, SEM	(*96*)
51.	*Quercus* sp.	Copper Sulfate ($CuSO_4$)	Whole plant	20–30	Quasi-spherical	FE-SEM, XRD, FTIR	(*97*)
52.	*Ziziphus mauritiana* Lam.	Copper Sulfate ($CuSO_4$)	Whole plant	20–45	Spherical	XRD, SEM, EDX, TEM	(*81*)
53.	*Ferulago angulata* (Schltdl.) Boiss.	Copper (II) Acetate Monohydrate ($Cu(CH_3COO)_2.H_2O$)	Whole plant	44 (in avg.)	Spherical	XRD, SEM, EDX, TEM	(*98*)
54.	*Gloriosa superba* L.	Copper Sulfate ($CuSO_4$)	Whole plant	5–10	Spherical	XRD, SEM, EDX, TEM	(*99*)
55.	*Syzygium alternifolium* (Wight) Walp.	Copper Sulfate ($CuSO_4$)	Bark	17.2 (in avg.)	Spherical	UV-Vis, XRD, FTIR, DLS, Zeta, TEM	(*100*)
56.	*Zea mays* L.	Copper Sulfate ($CuSO_4$)	Dry husk	36–73	Spherical	XRD, HR-TEM, EDX, FTIR	(*101*)
57.	*Caesalpinia bonducella* (L.) Fleming	Copper Sulfate ($CuSO_4$)	Seeds	13.07 (in avg.)	Spherical	UV-Vis, XRD, FTIR, and SEM	(*102*)
58.	*Vitis vinifera* L. (Vernacular name: Erzincan Cumin)	Copper Sulfate ($CuSO_4$)	Fruits	25–50	Uniform spherical	UV-Vis, FTIR, XRD and SEM	(*103*)
59.	*Cedrus deodara* (Roxb. ex D.Don) G.Don	Copper Sulfate ($CuSO_4$)	Aqueous extract	16	Spherical	FTIR, UV-Vis, XRD, TEM	(*104*)
60.	*Abutilon indicum* (L.) Sweet	Copper (II) Nitrate Trihydrate ($Cu(NO_3)_2.3H_2O$)	Leaves	16.78	Spherical	XRD, EDX, UV-Vis, SEM	(*105*)
61.	*Aloe barbadensis* Mill.	Copper Sulfate ($CuSO_4$)	Leaves	20 (in avg.)	Spherical	UV-Vis, SEM, TEM, XRD, FTIR	(*106*)
62.	*Ficus religiosa* L.	Copper Sulfate ($CuSO_4.5H_2O$)	Leaves	577 (in avg.)	Spherical	FE-SEM, UV-Vis, XRD, FTIR, DLS	(*107*)
63.	*Phoenix dactylifera* L.	Copper Sulfate ($CuSO_4$)	Leaves	20–28	Spherical	UV-Vis, FTIR, XRD, SEM, and EDAX	(*108*)
64.	*Centella asiatica* (L.) Urb.	Copper Chloride ($CuCl_2.2H_2O$)	Leaves	5 (in avg.)	Spherical	UV-Vis, IR, EDX	(*109*)
65.	*Azadirachta indica* A.Juss.	Copper Chloride ($CuCl_2{\cdot}2H_2O$)	Leaves	38 (in avg.)	Spherical	UV-Vis, FTIR, XRD, SEM, EDX, DLS and TEM	(*110*)

(*Continued*)

Table 1. Continued.

Sl. No.	Plants/ plant extract	Precursor	Part used	Size of NPs (in nm)	Shape / structure / morphology	Characterization techniques used	Reference
66.	*Drypetes sepiaria* (Wight & Arn.) Pax & K.Hoffm.	Cupper Nitrate ($Cu(NO_3)_2.3H_2O$)	Leaves	298 (in avg.)	Spherical	UV-Vis, IR, XRD, and TEM, FTIR	(*111*)
67.	*Enicostemma littorale* Blume	Copper Sulfate ($CuSO_4$)	Leaves	30 (in avg.)	Spherical	UV-Vis, IR, XRD, and TEM, FTIR	(*112*)
68.	*Cordia myxa* L.	Copper Sulfate ($CuSO_4.5H_2O$)	Leaves	20–106	Spherical	XRD, TEM, FTIR	(*113*)
69.	*Arachis hypogaea* L.	Copper (II) Aetate Monohydrate	Leaves	30–50	Spherical	UV-Vis, XRD, FTIR, and SEM	(*114*)
70.	*Leucaena leucocephala* (Lam.) de Wit	Copper Acetate Monohydrate ($Cu(CH_3COO)_2.H_2O$)	Leaves	10–15	Spherical	UV-Vis, XRD, FTIR, and SEM, BET	(*115*)
71.	*Piper betle* L.	Copper Sulfate ($CuSO_4$)	Leaves	50–100	Spherical	XRD, SEM, EDX, TEM	(*116*)
72.	*Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.	Copper Sulfate ($CuSO_4$)	Leaves	48 (in avg.)	Spherical	UV-Vis, FTIR, XRD, EDAX, TEM and SEM	(*117*)
73.	*Ailanthus altissima* (Mill.) Swingle	Copper Sulfate ($CuSO_4$)	Leaves	20 (in avg.)	Spherical	UV-Vis, SEM, TEM, FTIR	(*118*)
74.	*Saraca indica* L.	Copper Chloride ($Cu\ Cl_2.2H_2O$)	Leaves	40–70	Spherical	UV-Vis, XRD, EDX, FTIR, XPS, SEM, HR-TEM, TEM and SAED	(*119*)
75.	*Spinacia oleracea* L.	Copper Sulfate ($CuSO_4.5H_2O$)	Leaves	1–12	Spherical	XRD, TEM	(*120*)
76.	*Eclipta prostrata* (L.) L.	Copper acetate	Leaves	23–57	Spherical	UV-Vis, FTIR, XRD, SEM, HR-TEM, EDS	(*121*)
77.	*Cassia auriculata* L.	Copper Sulfate ($CuSO_4$)	Leaves	23 (in avg.)	Spherical	FTIR, UV-Vis, XRD, TEM, DLS	(*122*)
78.	*Solanum lycopersicum* L.	Copper Sulfate ($CuSO_4.5H_2O$)	Leaves	20–40	Spherical	UV-Vis, FTIR, FE-SEM, HR-TEM, XRD, DLS	(*123*)
79.	*Populus ciliata* Wall. ex Royle	Copper Nitrate Hexahydrate ($Cu(NO_3)_3.6H_2O$)	Leaves	50–60	Spherical	FTIR, UV-Vis, EDX, SEM, XRD, TEM	(*124*)
80.	*Bauhinia tomentosa* L.	Copper Sulfate ($CuSO_4$)	Leaves	22–40	Spherical	UV-Vis, XRD, TEM, EDX, FTIR	(*81*)
81.	*Alternanthera sessilis* (L.) R.Br. ex DC.	Copper Sulfate ($CuSO_4$)	Leaves	22.6-25.2	Spherical	SEM- EDAX, FTIR, XPS	(*81*)
82.	*Citrofortunella microcarpa* (Bunge) Wijnands	Copper Sulfate ($CuSO_4$)	Leaves	54–68	Spherical	UV-Vis, FTIR, XRD, SEM and EDS	(*125*)
83.	*Olea europaea* L.	Copper Sulfate ($CuSO_4$)	Leaves	20–50	Spherical	UV-Vis, FTIR, XRD, TEM, and SEM	(*125*)
84.	*Adiantum lunulatum* Burm. f.	Copper Sulfate ($CuSO_4$)	Whole Plant	1–20	Quasi-spherical	UV-Vis, DLS, Zeta Potential, FTIR, XRD, TEM and EDX	(*54*)
85.	*Sida acuta* Burm.f.	Copper Sulfate ($CuSO_4$)	Leaves	50	Crystalline	SEM,TEM, FTIR,Single beam spectroscopy,UV-vis	(*126*)

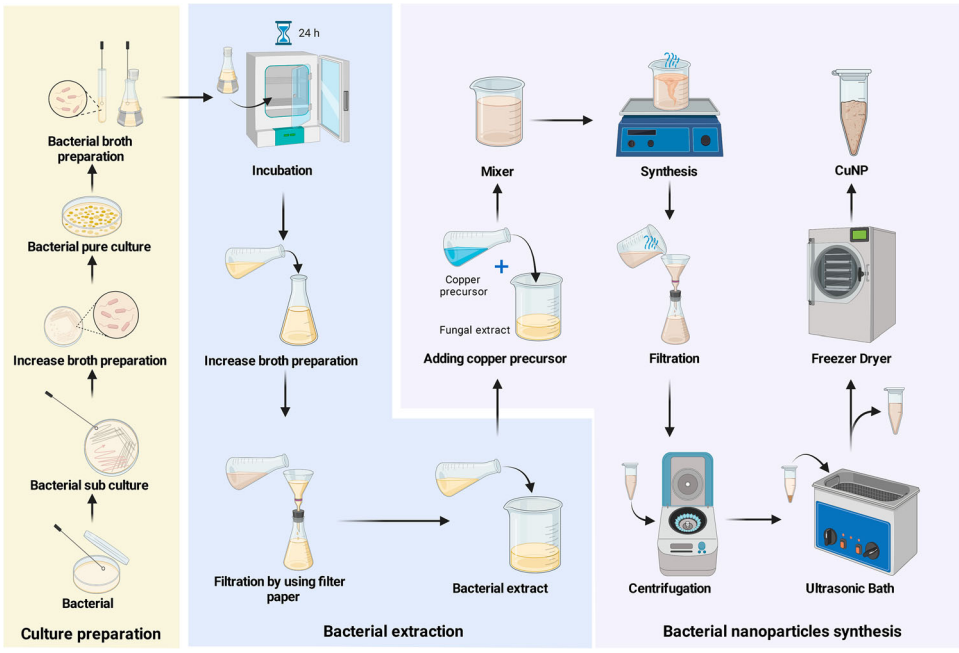


Figure 3. Graphical representation of biomediated synthesis of copper oxide nanoparticles using bacteria (Created with BioRender.com).

other growth chamber. For the biogenic production of nanoparticles, cell-free extracts of microorganisms, function as reducing, catalytic, or capping agents (*127*). *Trichoderma* species produce a wide range of bioactive metabolites, including pyrones, polyketides, terpenes, diketopiperazine, glycolipids, and a large number of reductive enzymes that aid in the production of not just CuO nanoparticles but also Ag and ZnO nanoparticles (*152–154*). Fungi use both internal and external routes to create various nanoparticles. The size of nanoparticles produced inside fungal species may be smaller than those produced by the extracellular pathway, with superior dispersity and dimensions. (*155*). Nanoparticle fabrication via the extracellular route provides several advantages. The nanoparticles created might be devoid of cell components. The extracellular route of fungus has mostly been used to synthesize nanoparticles because fungi secrete several types of metabolites that function as reducing and stabilizing agents for nanoparticle formation (*154*). Metal oxide nanoparticles, particularly copper oxide nanoparticles, have been synthesized using several fungus strains.

So, in this endeavor, several different species of fungus were studied, and it was discovered that fungi are excellent candidates since they release huge amounts of enzymes and are easier to work within the laboratory. *Penicillium aurantiogriseum*, *Penicillium citrinum*, and *Penicillium waksmanii* all produce CuNPs extracellularly (*156*). Majumder (*157*) described the production of CuNPs from *Fusarium oxysporum* at room temperature, which was then screened for copper extraction from integrated circuits and produced in nano form. Dead biomass of *Hypocrea lixii* recovered from the metal mine was used to manufacture spherical CuNPs with an average size of 24.5 nm, and an infrared spectroscopy investigation was conducted, they discovered that amide groups in proteins were responsible for the CuNPs stability and capping agents (*158*). Some of the contributions of biomediated synthesis of copper oxide nanoparticles using different fungi are shown in Table 3 (Figure 4).

2.5. Green synthesis of Cu/CuO-NPs by using algae

Algal members have gained importance in the synthesis of CuONPs when these nanoparticles with sizes ranging from 5 to 45 and 6 to 7.8 nm have been effectively produced by utilizing a boiling aqueous extract from the brown algae *Bifurcaria bifurcata* (*170*) and *Cystoseira trinodis* (*171*) respectively. Ramaswamy et al. (*172*) also employed an aqueous extract from brown seaweed (*Sargassum polycystum*) to make CuONPs. An autoclaved aqueous extract from the green microalgae *Botryococcus braunii* generated CuONPs with sizes ranging from 2–10 nm (*173*). Alternatively, Bhattacharya et al. (*174*) used a slightly different technique, heating the extract at 50°C rather than boiling it, to get an aqueous extract from the microalgae, *Anabaena cylindrica* and

Table 2. Biosynthesis of Cu/CuO nanoparticles by bacteria.

Sl No.	Bacterial Culture	Gram Nature	Precursor	Mode of Synthesis	Size of NPs (in nm)	Shape / Structure / Morphology	Characterization techniques used	Reference
1.	*Escherichia coli* (Migula) Castellani and Chalmers	Gram-negative	Copper Sulfate ($CuSO_4$)	Extracellular	100–150	Quasi-spherical	TEM, SEM, XRD, FTIR	(*135–137*)
2.	*Mycobacterium psychrotolerans* Trujillo and *Morganella morganii RP42* Winslow	Gram-negative	Copper Sulfate ($CuSO_4$)	Extracellular	15–20	Quasi-spherical cubic	SPR, FFT, UV-Vis, XPS, HR-TEM, LSV	(*138*)
3.	*Pseudomonas fluorescens* Migula	Gram-negative	Copper Sulfate ($CuSO_4$)	Extracellular	20–80	Spherical and hexagonal	TEM, SEM, EDS, UV-Vis, SPR	(*139*)
4.	*Pseudomonas stutzeri* (Lehmann and Neumann) Sijderius	Gram-negative	Copper Sulfate ($CuSO_4$)	Extracellular	8–15	FCC, spherical	TEM, SEM, EDS, UV-Vis, SPR	(*140*)
5.	*Pseudomonas stutzeri* (Lehmann and Neumann) Sijderius	Gram-negative	Copper Sulfate ($CuSO_4$)	Extracellular	50–150	FCC, cubic	TEM, SEM, EDS, UV-Vis, SPR	(*141*)
6.	*Serratia* sp.	Gram-negative	Copper Sulfate ($CuSO_4$)	Extracellular	10–30	Polydisperse	TEM, SEM, EDS, UV-Vis, SPR, EDX analysis	(*142*)
7.	*Streptomyces* sp.	Gram-negative	Copper Sulfate ($CuSO_4$)	Extracellular	100–150	Spherical	TEM, SEM, EDS, UV-Vis, SPR, EDX analysis	(*143*)
8.	*Streptomyces cyaneus* (Krassilnikov) Waksman	Gram-positive	Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	Extracellular	29.8	Spherical	UV-Vis, DLS, FTIR, TEM, XRD	(*136, 144*)
9.	*Shewanella loihica* PV-4	Gram-negative	Copper (II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$)	Extracellular	6–20	FCC, cubic	TEM, EDX, XRD, XPS	(*145*)
10.	*Salmonella typhimurium*	Gram-negative	Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	Extracellular	49	Spherical	UV-Vis, DLS, SEM	(*146*)
11.	*Bacillus cereus*	Gram-positive	Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	Extracellular	11–33	Spherical	UV-Vis, DLS, Zeta, FTIR, EDX, SEM, TEM, AFM, XRD	(*147*)
12.	*Streptomyces sp.* MHM38	Gram-positive	Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	Extracellular	1.72–13.49	Spherical	UV-Vis, EDX, TEM, XRD	(*148*)
13.	*Lactobacillus casei* subsp. *casei*	Gram-positive	Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	Extracellular	30–75	Spherical	FTIR, XRD, FESEM, TEM	(*149*)
14.	*Morganella* sp.	Gram-negative	Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	Extracellular	15–20	Spherical	UV-Vis, TEM, HR-TEM, XPS	(*150*)
15.	*Actinomycetes*	Gram-positive mycelial	Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	Extracellular	61.7	Spherical	UV-Vis, DLS, Zeta, FTIR, EDX, SEM, TEM, XRD	(*151*)

effectively able to generate CuONPs from that extract with a particle size of 3.6 nm.

Thus, the numerous algal members responsible for the reduction and stabilization process using copper as a promotor, as well as their diverse organic components, have yet to be identified in detail (Figure 5). As a result, it's vital to focus research on the usage of biomolecules in green synthesis on CuONPs to expand their biological applications. These are listed in Table 4.

3. Characterization of CU/CUONPs

To establish the demand for the generation of nanoparticles one researcher should go through a series of characterization. After the synthesis of NPs, the crystal structure and chemical composition are the initial stage in the characterization process (*177*). The size and morphology of the Cu/CuONPs were investigated using scanning electron microscopy, transmission electron microscopy, dynamic light scattering, particle analyzers, and field emission scanning electron microscopy, while UV–visible spectroscopy, X-ray diffraction, Fourier transform infrared spectroscopy, surface plasmon resonance, and energy-dispersive X-ray spectroscopy were used to analyze the elemental chemical compositions of Cu/CuONPs (Figure 6) (*178*).

4. Applications of copper nanoparticles (CUNPs)

CuNPs have diverse scientific applications. They are very effective against different pathogenic microbes. A high concentration of CuNPs generates reactive oxygen species in bacterial cells which eventually cause cell lysis. Moreover, CuNPs have exhibited anticancer and antifungal activities. Due to their antimicrobial activity,

Table 3. Biosynthesis of Cu/CuO nanoparticles by fungi.

Sl. No.	Fungal Culture	Precursor	Mode of Synthesis	Size of NPs (in nm)	Shape / Structure / Morphology	Characterization techniques used	Reference
1.	*Fusarium oxysporum* Schltdl.	Metallic copper	Extracellular	93–115	–	TEM, SEM, UV- Vis	(*157*)
2.	*Hypocrea lixii* Pat.	Metallic copper	Extracellular	24.5 (in avg.)	Spherical	TEM, SEM, UV-Vis	(*158*)
3.	*(a) Penicillium aurantiogriseum* Dierckx			89–250	Spherical	TEM, SEM, DLS, UV-Vis, FTIR, AFM	(*156*, *159*)
	(b) Penicillium citrinum Thom	Copper Sulfate ($CuSO_4$)	Extracellular	85–295	Spherical		
	(c) Penicillium waksmanii K.W. Zaleski			79–179	Spherical		
4.	*Stereum hirsutum* (Willd.) Pers.	Copper salts ($CuSO_4$, $CuCl_2$)	Extracellular	4–5	monodispersed, spherical	TEM, FTIR, XRD, and Zeta Potential	(*160*)
5.	*Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison	$CuCl_2$	Extracellular	10.5 (in avg.)	Spherical		(*158*)
6.	*Aspergillus niger* Tiegh.	Copper Sulfate ($CuSO_4$)	Extracellular	5–100	Spherical	TEM, SEM, DLS, UV-Vis, FTIR	(*161*)
7.	*Trichoderma harzianum* Rifai	Copper (II) Sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$)	Extracellular	5–18	Dense agglomerate and spherical	TEM, SEM, DLS, UV-Vis, XRD	(*154*)
8.	*Aspergillus flavus* Link	Copper (II) Sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$)	Extracellular	20 (in avg.)	Spherical	TEM, UV-Vis, XRD, FTIR, NTA	(*162*)
9.	*Trichoderma asperellum* Samuels, Lieckf. & Nirenberg	Copper nitrate ($Cu(NO_3)_2.3H_2O$)	Extracellular	110 (in avg.)	Spherical	FETEM, HR SEM	(*163*)
10.	*Aspergillus fumigatus* Fresen.	Copper nitrate ($Cu(NO_3)_2.3H_2O$)	Extracellular	8 (in avg.)	Spherical	UV-Vis, HRTEM, Zeta, FTIR, XRD, EDX	(*164*)
11.	*Neurospora crassa* Shear & B.O. Dodge	Copper(II) chloride ($CuCl_2$)	Extracellular	10–20	Spherical	TEM, SEM, FTIR, EDX, XRD	(*165*)
12.	*Pestalotiopsis* sp.	Copper(II) chloride ($CuCl_2$)	Extracellular	10–20	Spherical	TEM, SEM, FTIR, EDX, XRD	(*165*)
13.	*Myrothecium gramineum* Lib.	Copper(II) chloride ($CuCl_2$)	Extracellular	10–20	Spherical	TEM, SEM, FTIR, EDX, XRD	(*165*)
14.	*Alternaria alternata* (Fr.) Keissl.	Copper (II) Sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$)	Extracellular	60–80	Spherical	TEM, EDX, XRF, SDD	(*166*)
15.	*Botrytis cinerea* Pers.	Copper (II) Sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$)	Extracellular	60–80	Spherical	TEM, EDX, XRF, SDD	(*166*)
16.	*Aspergillus oryzae* (Ahlb.) Cohn	Copper (II) Sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$)	Extracellular	55	Spherical	UV–Vis., TEM, DLS, XRD, EDX, SEM and FT-IR	(*81*)
17.	*Aspergillus terreus* (Ahlb.) Cohn	Copper (II) Sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$)	Extracellular	15.75	Spherical	UV–Vis, FT-IR, XRD, TEM and SEM	(*167*)
i.	*Penicillium chrysogenum* Thom	Copper (II) Sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$)	Extracellular	9.70	Spherical	UV–Vis., XRD, FTIR, DLS, EDX TEM, SEM	(*168*)
ii.	*Pleurotus ostreatus* (Kalchbr.) Pilát	Copper nitrate ($Cu(NO_3)_2.3H_2O$)	Extracellular	10–190	Spherical	UV–Vis, TEM, DLS, XRD, XRD and FT-IR	(*169*)

it is also used for food preservation and agricultural field to draw protection against different pathogenic fungi and bacteria (*179*). Copper-based nano fertilizer and nano-insecticides promote growth and nutrients in crop plants. Copper-based bioremediation plays a pivotal role in waste-water treatment and removal of heavy metals from soil. Copper is a good conductor of electricity hence it is used as a super-conductor and has a significant contribution to the modern electronic field (*180*). In Figure 7, we have summarized the different applications of CuNPs and also elaborate its major application in the following context.

4.1. Antimicrobial activity

In the past few decades, metal and metal oxides nanoparticles are being used to treat different bacterial and viral diseases. Nanoparticles based antibiotics and other drugs gained a special attraction since it is less toxic, eco-friendly and exhibited potential disease curing activity. Several reports demonstrated that Cu and CuONPs have tremendous antimicrobial activities against different pathogenic microbes. High concentrations of CuNPs are toxic to the different bacterial pathogens of both humans and plants (*181*). CuNP has

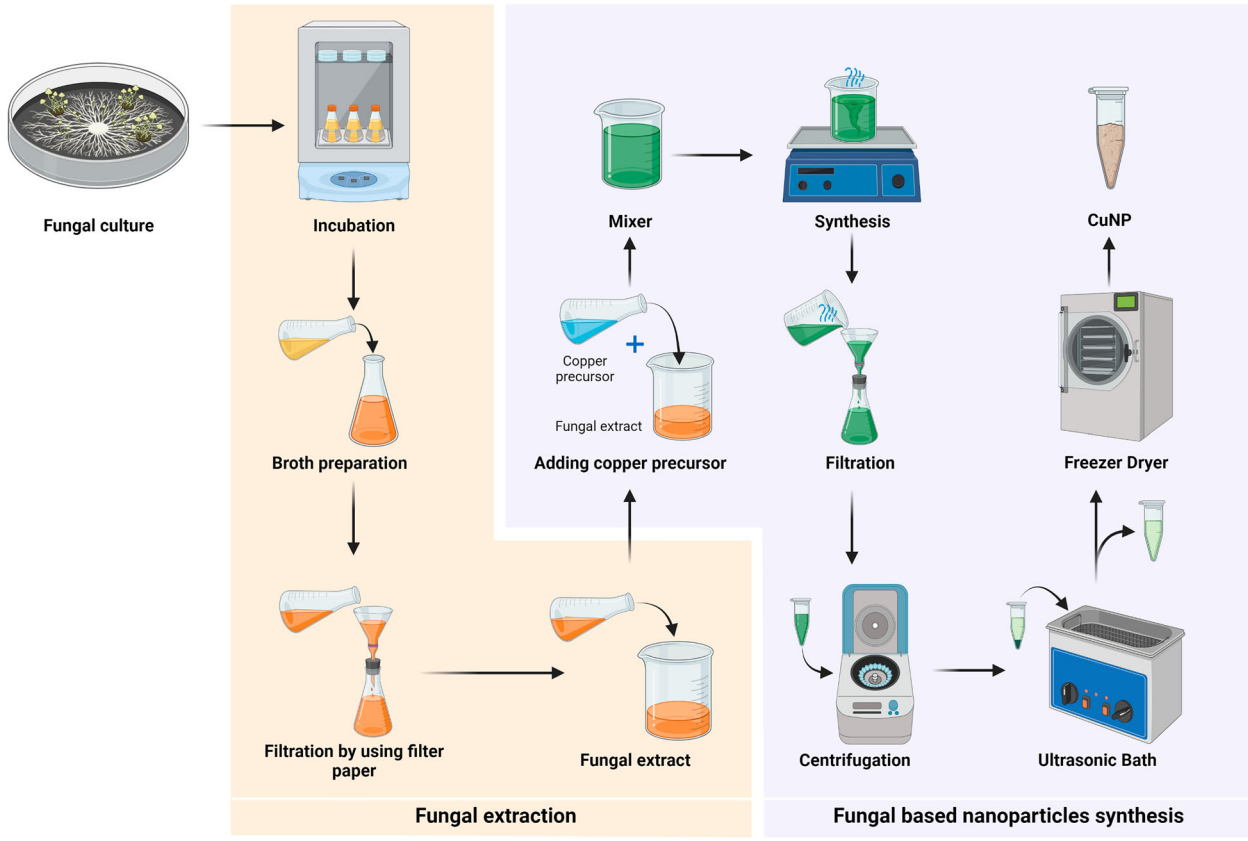


Figure 4. Graphical representation of biomediated synthesis of copper oxide nanoparticles using different fungi (Created with BioRender.com).

some unique features like small size, high surface area, biocompatibility, high biological and chemical reactivity which helps to kill bacterial cells efficiently. Bio fabricated CuNPs possess antimicrobial activity against both gram-positive and gram-negative pathogenic bacterial strains (*182*). Green synthesized Cu and CuONPs exhibited potential antibacterial activity against *Pseudomonas aeruginosa*, *Clostridium difficile*, *Staphylococcus aureus* and *Escherichia coli* (*36, 81*). The fabrication of CuNPs using *Gloriosa superba* leaf extract showed inhibition against gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Klebsiella aerogenes* (*179*). Green synthesized CuONP using *Sida acuta* leaf extracts can be used commercially in the textile industry as a potential antimicrobial agent. It was found that *S. acuta* coated CuONPs can inhibit both Gram positive and Gram negative bacteria when it was applied in the cotton fabrics (*126*). Besides antibacterial activity, the antiviral activity of green synthesized CuNP has also been confirmed. Green synthesized CuNPs using clove fruit extract can inhibit the Newcastle disease virus (*183*). In the following table, we have listed the application of CuNPs as an anti-microbial agent against different microbes (Table 5).

When bacterial cells come in contact with CuNPs, it develops toxicity inside the bacterial cell which leads to several malfunctions and ultimately kills the cells. Due to the small particle size of the CuNPs it can easily take entry inside the bacterial cell through the cell membrane. The carboxylic and amines group present in the bacterial cell membrane helps to attract the Cu ions efficiently. The toxicity of CuNPs greatly varies with the size and shape of the particles. (*192*). CuNPs accumulates reactive oxygen species which can disrupt the cell membrane and provide direct cellular toxicity (*185*). Copper has a great redox potential which can act as an electron donor or acceptor by producing Cu ions. These ions are very toxic for bacterial cells and accumulate superoxides and hydroxyl radicals leading to oxidative stress. These ROS generation can interfere with the cellular process of bacteria like DNA replication, cell division and metabolism (*36*). CuNP mediated toxicity in bacterial cells promotes degradation of mitochondria, ribosomes and different proteins channels present in the bacterial cell membrane. The exact mechanism of antimicrobial activity is still under study. A probable antimicrobial mechanism of CuNPs is presented in the following diagram (Figure 8).

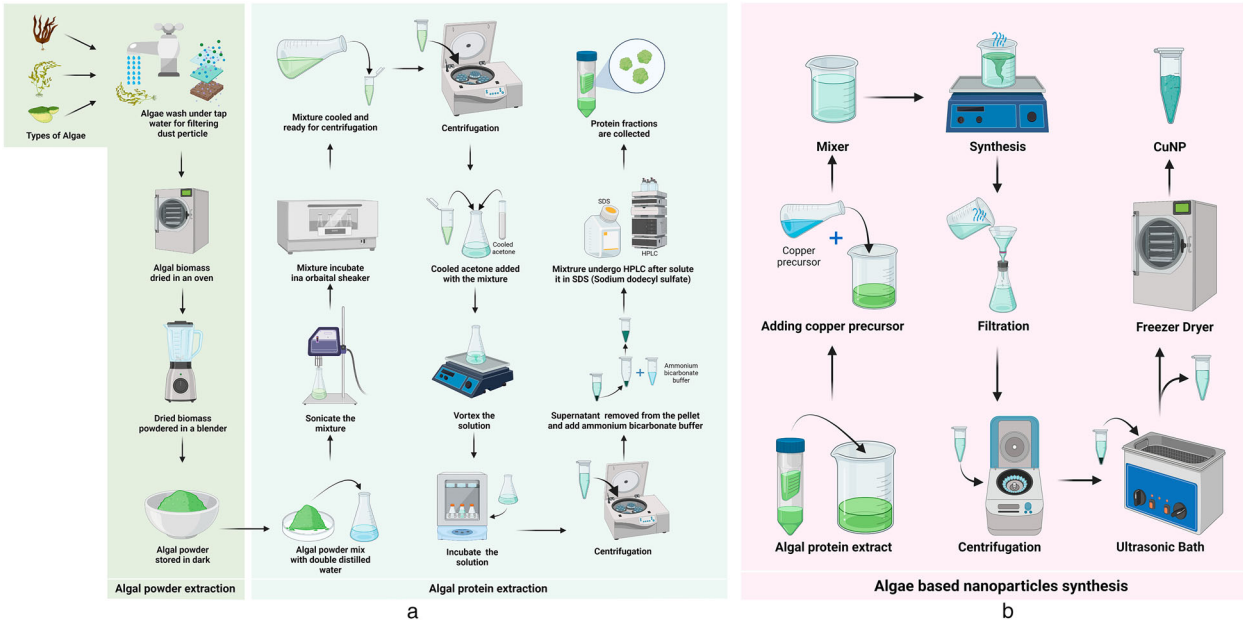


Figure 5. (a) Graphical representation of the preparation of the algal protein extract for the synthesis of copper oxide nanoparticles using different algae (Created with BioRender.com) (b) Graphical representation of biomediated synthesis of copper oxide nanoparticles using different algae protein extract (Created with BioRender.com).

4.2. Antifungal activity and crop protection

In the current medical sector, the fungal disease has become a severe health threat and many patients die every year especially those with a poor immune system. Though there are several antifungal drugs are available in the market, but the development of drug resistance is a severe threat. Several studies revealed that CuNPs can be used as an antifungal agent since they can inhibit several pathogenic fungi in both humans and plants (*193*). However, more studies are needed to ensure the exact antifungal mechanisms of CuNPs and their future use as an antifungal agent. Green synthesized CuNPs can control several pathogenic fungal strains like *Fusarium oxysporum* Schltdl., *Alternaria solani* (Ellis & G. Martin) L.R. Jones, *Aspergillus niger* Tiegh., and *Penicillium citrinum* Thom (*194*). CuNPs synthesized by using Chitosan exhibited antifungal activity against tomato plant pathogen *A. solani* and *F. oxysporum* (*195*). Chemically synthesized CuNPs are highly effective against *Candida albicans* (C.P. Robin) Berkhout and *Aspergillus flavus* Link (*196*). In another report CuNPs exhibited antifungal activity against some destructive crop pathogens like *Alternaria alternata* (Fr.) Keissl., *Curvularia lunata* (Wakker) Boedijn and *Phoma destructiva* Plowr. (*197*). Green synthesized CuNPs using *Syzygium alternifolium* (Wight) Walp. are known to have antifungal activity against some plant pathogens. CuNPs can be used in the formulation of nanofungicides. In a field study, it was found that Cu based fungicides are more effective than other agrochemicals against tomato pathogen *Phytophthora infestans* (Mont.) de Bary (*198*). Since CuNP has potential antimicrobial and antifungal properties it could be

Table 4. Biosynthesis of Cu/CuO nanoparticles by algae.

Sl No.	Algal extract	Precursor	Size of NPs (in nm)	Shape/ Structure / Morphology	Characterization techniques used	Reference
i.	*Bifurcaria bifurcata* R. Ross	Copper Sulphate solution ($CuSO_4.5H_2O$)	5–45	Crystallite	UV-Vis, TEM, FTIR, XRD	(*170*)
ii.	*Cystoseira trinodis* (Forsskål) C. Agardh	Copper Sulfate ($CuSO_4$)	6–7.8	Crystallite	TEM, SEM, XRD, FTIR, EDX, Raman, UV-Vis	(*171*)
iii.	*Botryococcus braunii* Kützing	Copper Acetate Cu $(CH_3COO)_2$	10–70	Cubical and Spherical	UV-Vis, FTIR, SEM, X-Ray Diffraction.	(*173*)
iv.	*Anabaena cylindrica* Lemmermann	Copper Sulphate solution ($CuSO_4.5H_2O$)	3.6 (in avg.)	Crystallite	XRD, XPS, EDX	(*174*)
v.	*Macrocystis pyrifera* (L.) C.Ag.	Copper Sulfate ($CuSO_4$)	2–50	Spherical	DLS, Zeta Potential, FTIR, TEM, EDS	(*175*)
vi.	*Sargassum polycystum* C. Agardh	Aqueous copper	–	Spherical	TEM, SEM, XRD, FTIR, EDX, UV-Vis	(*176*)

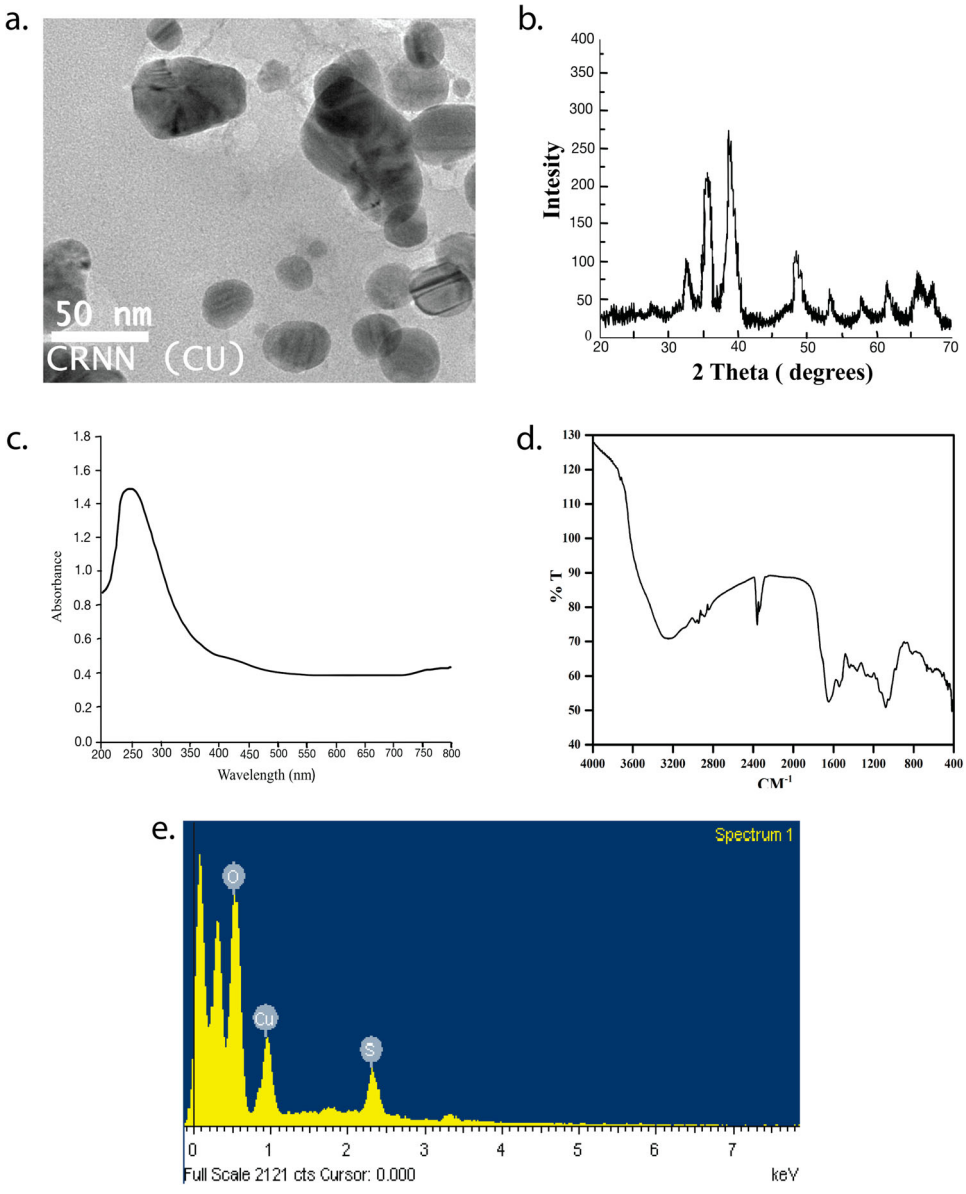


Figure 6. Characterization of synthesized copper oxide nanoparticles (CuONPs) (a) TEM images of CuONPs (b) XRD analysis of CuONPs (c) UV-Visible Spectroscopic analysis of CuONPs (d) FTIR analysis of CuONPs (e) EDX analysis of CuONPs.

used in food preservation and food packaging as well. High concentrations of copper provide direct toxicity to several food spoilage microbes and fungi (*81*). Some antifungal applications of CuNPs are listed below (Table 6).

4.3. Anticancer activity

Currently, cancer is the most dangerous and common disease which increases the mortality rate worldwide. Till now no promising drugs are available in the market to treat cancer. The most commonly used radiotherapy and chemotherapy have tremendous side effects and are also expensive processes. Several studies are still under process to discover an alternative nontoxic biological drug. In this regard, it was found that the emergence of nanotechnology helps to treat different types of cancer efficiently. Biologically synthesized Cu and CuO nanoparticles exhibited promising results when it tested against some human cancer cell lines (Table 7) (*201–207*).

Green synthesized CuNPs using dry black beans can inhibit the growth of human cervical carcinoma and also showed cytotoxicity against HeLa cell line by producing ROS (*204*). The mechanism of CuNP mediated anticancer activity includes oxidative stresses, accumulation of ROS, chromosomal aberration, genetic material fragmentation, production of caspases, enhancement of

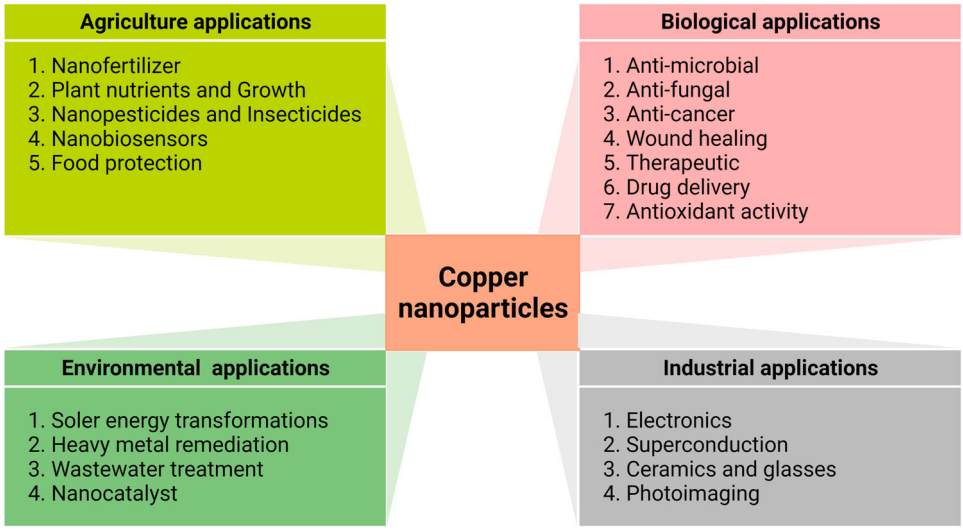


Figure 7. Applications of copper nanoparticles (Created with BioRender.com).

intrinsic and extrinsic apoptotic pathways (Figure 9) (*192*).

In a study, it was found that green synthesized CuONPs can inhibit the growth of A549 adenocarcinomic human alveolar epithelial cells. A high concentration of CuONPs promotes cellular toxicity and DNA damage in the A549 lung cells (*208*). In addition to this, it was also found that biologically synthesized chitosan/copper oxide nanocomposite using a bioflavonoid rutin, also exhibited antiproliferative efficiency when it was tested against A549 human lung cancer cell line (*209*). CuNP mediated anticancer activity has been observed in CaCO-2 human colon cancer cells, Mcf-7 breast cancer cells, HepG-2 hepatic cancer cells and HeLa cells (*210*). In a study, it was found that when HeLa cells are treated with copper oxide nanoparticles it showed oxdative stress mediated mitochondrial degradation. The normal control cells depicted by a normal mitochondrial structure whereas the treated cancer cells are characterized by a condensed clumped structure of mitochondria which leads to apoptosis of the cancer cells (*83*). When the similar study was done with HepG-2 hepatic cancer cell line it exhibited enhanced apoptosis by upregulating the tumor suppressor gene and down regulating the antiapoptotic gene bcl-2 (*211*).

To explain this finding, it is important to note that various factors, including size distribution, shape, and surface chemistry of biogenic nanoparticles, can influence their cytotoxicity. Different cytotoxicity responses may result from changes in various parameters. Furthermore, biomolecules are responsible for bioreduction of metal ions to their nano-forms in the biosynthetic method. These biomolecules are attached to the surface of biosynthesized nanoparticles and act

Table 5. Applications of green synthesized CuNPs as antimicrobial agent.

Sl. No.	Biological source	Chemical used	Antimicrobial Application	Reference
1.	*Eucalyptus globulus* Labill.	$CuSO_4$	It accumulates ROS inside the bacterial cells which lead to cell destruction.	(*137*)
2.	*Zea mays* L.	Cu $(CH_3COO)_2$	It is effective against some pathogenic bacteria like *Bacillus lichiniformis* and *Pseudomonas aeruginosa*.	(*184*)
3.	*Citrus medica* L.	$CuSO_4$	It can inhibit several crop pathogens and also inhibit the growth of *Propionibacterium acnes* and *Klebsiella pneumoniae*.	(*63*)
4.	*Camellia sinensis* (L.) Kuntze	$Cu(NO_3)_2$	It can inhibit the growth of *Vibrio cholerae* and *Klebsiella pneumonia*.	(*185*)
5.	*Bacillus cereus* Frankland and Frankland	$CuSO_4$	It can control the growth of pathogenic bacteria like *P. aeruginosa, Staphylococcus aureus, E. coli* and *Bacillus subtilis*.	(*186*)
6.	*Nerium oleander* L.	$CuSO_4$	Antibacterial activity against *Salmonella typhi*, *E. coli, Bacillus subtilis* and *Staphylococcus aureus*.	(*187*)
7.	*Sida acuta* Burm.f.	$CuSO_4$	Exhibited tremendous antimicrobial activity against some human pathogens and also effective against bacterial contamination in the textile industries.	(*126, 188*)
8.	*Punica granatum* L.	$CuSO_4$	Exhibited antimicrobial activity against *Micrococcus luteus*, *Salmonella enteric* and *Enterobacter aerogenes*.	(*189*)
9.	*Pseudomonas fluorescens* Migula	$CuSO_4$	Inhibit the growth of *Bacillus* and *E. coli*.	(*134*)
10.	Chitosan derived from fungal cell wall	$CuSO_4$	Effective against *Salmonella paratyphi*.	(*190*)
11.	*Ocimum sanctum* L.	$CuSO_4$	Antimicrobial activity against human pathogenic microbes.	(*191*)

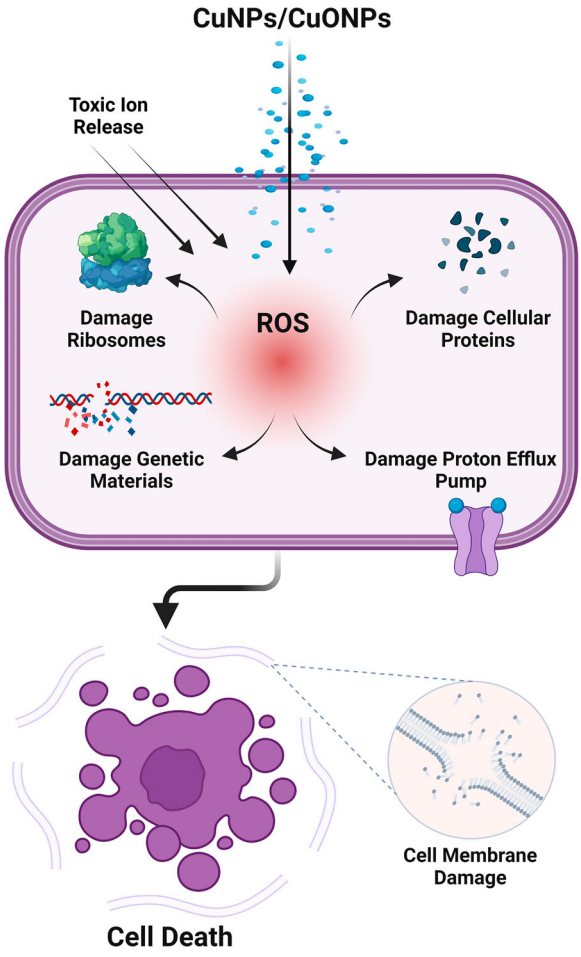


Figure 8. Probable antimicrobial mechanism of Cu/CuONPs (Created with BioRender.com).

as a stabilizer, preventing the nanoparticles from aggregating. These biomolecules adhering to the surface of the nanoparticles, have the potential to alter the surface chemistry of various nanoparticles and interfere with their ability to respond to their biological environment. As a result, the biological sources used to make nanoparticles may have an impact on their cytotoxicity response (*212–215*).

4.4. Plant growth, nutrients and defense booster

Plants need to uptake different minerals and nutrients elements for their proper growth and development. Plants nutrients are divided into two classes macronutrients and micronutrients. Macronutrients are generally required in high concentrations and micronutrients are required comparatively in low concentrations. Both these macro and micronutrients are essential for maintaining structural integrity and normal growth in plants. Deficiency of which causes disease and death of different plant parts (*81*, *216*). In plants, copper is required in very low concentrations as it is a micronutrient. A high concentration of Cu promotes toxicity and hampered growth in plants. Plant chloroplast contains a maximum number of Cu as it helps in chloroplast and other pigments synthesis. Cu deficiency leads to several abnormal conditions like young leaf distortion, necrosis, stem bending, affects vegetative growth and reduces grain quality in crop plants (*217*). CuNP mediated plant growth responses depend upon several factors like concentration, size, plant species and structure of the particles. In a study, it was found that CuNPs can stimulate root and shoot growth in *Phaseolus radiates* and *Triticum aestivum*. The growth response typically varies with varying concentrations of CuNPs. When wheat plants were treated with 20, 25, 30 and 35 ppm concentrations of CuNPs it showed better growth and yields. Concentrations above 1000 ppm reduced growth in wheat followed by a decrease in yields (*218*). When CuNPs were applied on *Allium cepa* with 20 µg/ml concentrations it enhanced growth and mitotic index. The mitotic index decreased with increasing concentrations of CuNPs (*219*). Applications of CuONPs increased shoot and root growth in *Zea mays* (*220*). Application of CuONPs on transgenic cotton plants enhanced expression of exogenous genes which code for Bt toxins in leaves (*221*). On the contrary high concentration of CuNPs has a negative

Table 6. Antifungal activity of different green synthesized CuNPs.

Sl. No.	Biological source	Chemical used	Antifungal Application	Reference
1.	*Cissus quadrangularis* L.	Cu $(CH_3COO)_2$	Inhibit the growth of pathogenic fungi *Aspergillus flavus* Link and *A. niger* Tiegh.	(*81*)
2.	*Brassica juncea* (L.) Czern.	$CuSO_4$	Inhibit the growth of *Alternaria alternata*, *Phoma destructiva* and *Curvularia lunata* (Wakker) Boedijn.	(*81*)
3.	*Citrus medica* L.	$CuSO_4$	Effective against pathogenic fungi *Fusarium graminearum* Schwabe, *F. oxysporum* Schltdl. and *F. culmorum* (Wm.G. Sm.) Sacc.	(*63*)
4.	*Oxalis corniculata* L.	$CuSO_4$	Inhibit the growth of *A. alternata, Pythium ultimum* Trow and *A. niger* Tiegh.	(*199*)
5.	*Syzygium alternifolium* (Wight) Walp.	$Cu(NO_3)_2$	Effective against *Trichoderma harzianum* Rifai	(*183*)
6.	*Penicillium chrysogenum* Thom	$CuSO_4$	Antifungal activity against *A. solani* (Ellis & G. Martin) L.R. Jones, *A. niger* Tiegh. and *F. oxysporum* Schltdl.	(*194*)
7.	*Syzygium aromaticum* (L.) Merr. & L.M.Perry	$CuSO_4$	Exhibited antifungal activity against pathogenic fungi *A. niger* Tiegh. and *A. flavus* Link.	(*200*)

Table 7. Different anticancer properties of CuNPs.

Sl. No.	Biological source	Tested cell lines	Anticancer activity	Reference
1.	*Camellia sinensis* (L.) Kuntze	MCF-7	Promotes significant cytotoxicity against breast cancer cells.	(*174*)
2.	*Bacillus cereus* Frankland and Frankland	A549 CaCO-2 HCT 116	Inhibit the growth of the tested cancer cell line.	(*153*)
3.	*Ficus religiosa* L.	A459	Inhibit lung cancer cells by inducing ROS mediated apoptosis.	(*175*)
4.	*Phaseolus vulgaris* L.	HeLa	Inhibit the growth of human cervical cancer cells which includes ROS mediated apoptosis and mitochondrial malfunctions.	(*171*)
5.	*Syzygium alternifolium* (Wight) Walp.	MCF-7	Inhibit the growth of breast cancer cells by promoting direct cytotoxicity.	(*150*)
6.	*Cordia myxa* L.	AMJ-13 MCF-7	Inhibit breast cancer cell growth.	(*176*)
7.	*Calotropis procera* (Aiton) Dryand.	BHK 21 HeLa A549	Promotes apoptotic destruction of the tested cancer cell lines.	(*177*)
8.	*Lactobacillus casei*	AGS HT-29	Inhibit the growth of human gastric cancer cells and colorectal cancer cells by promoting cytotoxicity and apoptosis.	(*178*)

impact on plant growth. CuNPs with different concentrations ranging from 200 to 1000 mg/l exhibited a negative impact on the growth of *Raphanus sativus*, *Triticum aestivum*, *Lolium perenne* and *Phaseolus radiates* (*222*). Application of CuONPs at a higher concentration in *Arabidopsis thaliana* seedlings inhibited root and shoot growth and also decreased chlorophyll contents (*223*). Recently, we have shown that treatment of CuONPs significantly boost up the defense enzymes, total phenol and other defense parameters along with plant vigor in *Lens culinaris* through nitric oxide signaling pathway (*224*). Possible mechanism has been illustrated in Figure 10.

4.5. Wound healing

Several studies over the last few years have ensured that CuNPs can be used as wound healing agents providing protection against infections. Wounds and cuts on the body surface are the most common pathway through which different infectious microorganisms take entry into the body. To prevent infection, it is very necessary to remove microbes from the wound site. As discussed earlier, CuNPs has potential antimicrobial and antifungal activity so it may be used in wound healing purposes (*225*). At the site of the wound Cu stimulates the formation of new blood vessels which leads to angiogenesis. Besides this CuNPs also stimulates the expression

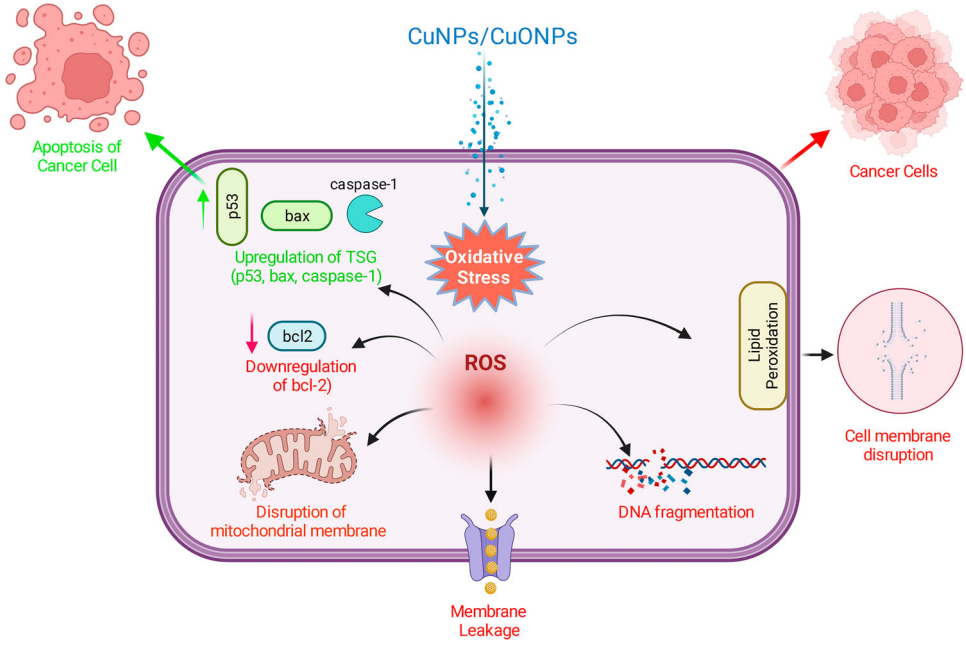


Figure 9. Probable anti-cancer mechanism of Cu/CuONPs (Created with BioRender.com).

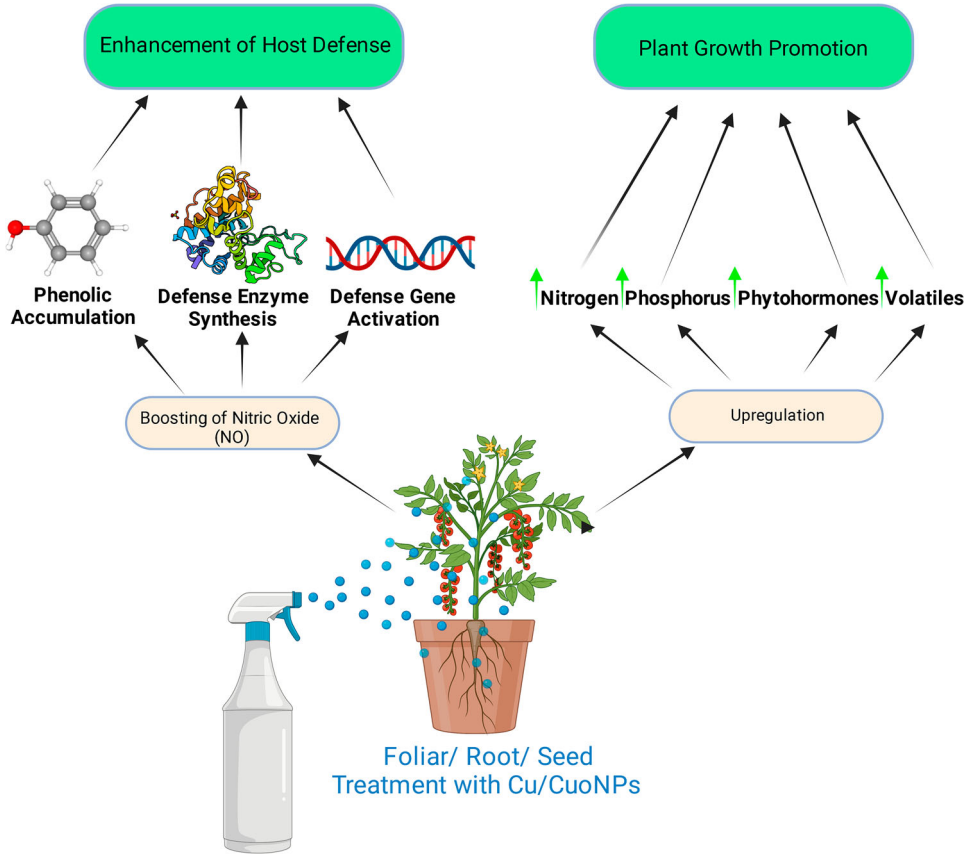


Figure 10. Schematic representation of defense induction and plant growth promotion by Cu/CuONPs (Created with BioRender.com).

of vascular endothelial growth factor (VEGF) that facilitates the transport of different nutrients and collagen formation necessary for repairing the wound (Figure 11). Application of Cu can halt severe phosphorus burn by the process of tissue remodeling. At the site of the wound Cu helps in maintaining the stability of fibrinogen and accumulates lysyl oxidase enzyme (*210*). Green synthesized CuNPs using *Falcaria vulgaris* leaf extracts exhibited potential cutaneous wound healing activity (*226*). In a study, a 500 mm cut was made on an anaesthetized animal. Three set up was made by applying biologically synthesized CuNP gel, nonbiologically synthesized CuNP and control. After some days it was found that biologically synthesized CuNPs were able to reduce 92% of the wound. Moreover, it promotes cell proliferation, cell migration and inhibits cyclooxygenase-2 enzyme at the wound site (*186*, *227*).

4.6. Other applications

CuNPs are now used in the textile industry for making antimicrobial personal protective equipment (PPE). In the fabric's polymer, nano-coppers are incorporated for making antimicrobial fabrics. CuNPs incorporated cotton fibers exhibited antimicrobial activities against several pathogenic microbes (*210*). CuNPs can be used to treat polluted water in different industrial areas. Several reports have described that CuNPs are used in the disposal of industrial waste and effluent. Besides bioremediation activity, CuNPs has a potential biocatalytic activity that can reduce and degrade xanthenes dyes, congo red, rhodamin-B, and methylene blue (*70*). Due to fluorescence quenching properties, CuNPs could be used as biosensing and biolabelling agent (*228*). Since CuNPs has a very small size it can interact with different biomolecules efficiently and can be used as drug delivering system (*179*). Green synthesized CuNPs exhibited larvicidal activities against *Aedes aegypti* providing protection against dengue, zika and chikungunya (*229*).

5. Future perspectives

In this era of nanotechnology, remarkable advancements are going on day by day in the field of nanoparticle synthesis and their sophisticated applications in the vast arena of science and technology. Beside others CuONPs/CuNPs have tremendous widespread applications, especially in biological systems. However, some nanosystems needs further sophistication and

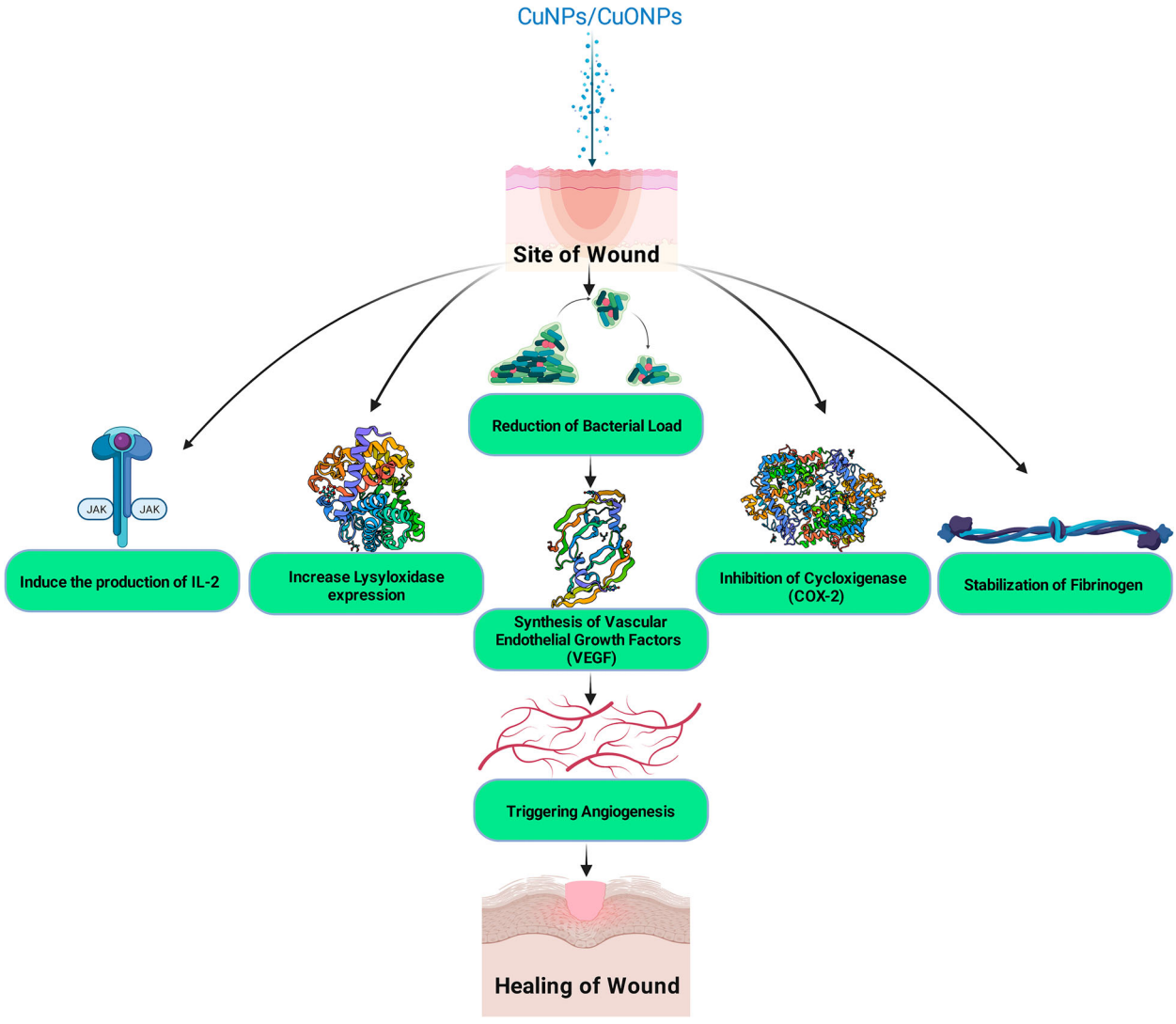


Figure 11. Probable wound healing mechanism of Cu/CuONPs (Created with BioRender.com).

they are still at the stage of infancy. More widespread research is still required to set the parameters. New cost-effective tools must be developed. Searching for new application of CuONPs/CuNPs has to be taken into consideration. Application of CuONPs/CuNPs in the research field of nano-sensors in film packaging of food, detection of microorganisms and toxic substances to check food quality etc. will give future directions. However, challenges and safety aspects can be taken as a serious concern. The findings of this review expand the prospects for the green synthesis of CuONPs/CuNPs and their use in a variety of biological and biotechnological domains.

6. Conclusion

In this present situation of global pollution and environmental consciousness it is utmost important to reduce the use of hazardous compounds in various technical fields of application-based research. In this context, it is evident to utilize CuONPs/CuNPs as a hazard free compound, at various important sectors of research like drug delivery system, in solving various health issues, plant defence booster, textile industry, etc. However, synthesis part is very important as harmful substances are used in both physical and chemical methods of CuONPs/CuNPs production. The biological technique, on the other hand, is eco-friendly, cost-effective, dependable, stable, uses little energy, and is a straightforward procedure. In this study, a comprehensive idea has been given on the pros and cons of bio-based synthesis and their characterization including their every possible application. However, to advance the biomedical applications of CuONPs, additional research should be conducted on ways to reduce the toxicity of CuONPs while preserving and enhancing their biological efficiency.

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Author contribution

J.S., N.C.: conceptualization; J.S., N.C., J.B., P.C.: methodology; J.S., N.C., J.B., A.B.: formal analysis and investigation; J.S., N.C., J.B., A.B., S.C., K.R.: writing–original draft preparation; P.C., J.S., J.B., N.C.: Image preparation; J.S., N.C.: Writing – review and editing; K.A. J.S., N.C.: supervision.

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